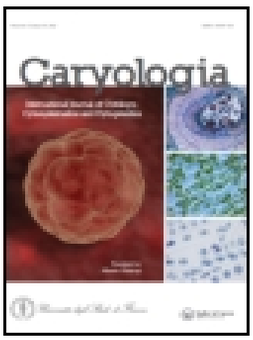

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Embryology of *Onobrychis persica* Sirj. and Rech.f. (Fabaceae) and its systematic implications

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ABSTRACT

Developmental aspects of anther, pollen grains, ovule, embryo and seed has described in *Onobrychis persica* Sirj. and Rech.f. (Fabaceae) under bright field, polarizing and fluorescence microscopy. Anther development starts when the flowers are very small. The anther is tetrasporangiate, and its wall development follows the dicotyledonous type and consists of four layers: epidermis, endothecium, middle layer and a secretory tapetum. Cytokinesis is simultaneous and arrangement of microspores is tetrahedral and tetragonal. Fibrous thickenings are developed in the endothecium when shed. Ellipsoidal tricolpate pollen grains are two-celled when anthers dehisce. The young hemianatropous ovule changes to a anatropous, crassinucellar and bitegumic mature one with zigzag micropyle. Meiosis of megasporocytes results in a T-shaped tetrad. The chalazal megaspore develops into an eight-nucleate embryo sac with the pattern of *Polygonum* type. The polar nuclei remain separated before fertilization. After cellularization of endosperm, peripheral cells show dense lipid content. The axial embryo shows fleshy cotyledons, which accumulate lipid and starch. The inner integument differentiates into an endothelium and largely vanishes during development while the outer one produces several layers and establishes the typical seed coat structure: macrosclereid cells, osteosclereids and parenchyma cells. Different compounds, such as starch and lipid content were demonstrated with special staining in the tissues. The systematic significance of the embryological characters is discussed in *O. persica*.

KEYWORDS

Hedysareae; embryo sac; female gametophyte; male gametophyte; *Onobrychis persica*; seed development

Introduction

The genus *Onobrychis* belonging to the tribe *Hedysareae*, from the Fabaceae family, with nearly 130 perennial and annual species, is mainly distributed in the Anatolia–Iran–Caucasian triangle (Arslan and Ertuğrul 2010). *Onobrychis* is an economically important genus used to improve the quality of the soil. It is also harvested as dried, fresh and purebred fodder (Arslan and Ertuğrul 2010).

This genus is subdivided into two subgenera, namely *Onobrychis* and *Sisyrosema* Bunge (Schischkin and Bobrov 1972; Rechinger 1984; Ahangarian et al. 2007; Duan et al. 2015). *Onobrychis* and *Sisyrosema* are represented by different karyotype characters, molecular investigations and geographical origins (Rechinger 1984; Hesamzadeh Hejazi and Ziaei Nasab 2010; Karamian et al. 2012; Ranjbar et al. 2010, 2012; Lewke Bandara et al. 2013).

In Iran, 54 species were treated under eight sections: *Dendrobrychis*, *Lophobrychis*, *Onobrychis*, *Laxiflorae*, *Anthyllium*, *Afghanicae*, *Heliobrychis* and *Hymenobrychis* (Rechinger 1984). There are many contradictions and uncertainty in the taxonomy of this genus, which is often confused with Hedysarum (Boissier 1872; Aktoklu 2001). Lewke Bandara et al. (2013) considered that the species delimitation of sect. *Onobrychis* is difficult.

O. persica, with the meiotic chromosome number of $2n = 2x = 14$ (Karamian et al. 2012), belongs to the *Onobrychis* section and is endemic in Iran. Embryological characters of this species are reported here for the first time.

Embryological studies in Papilionoideae (Ashrafunnisa and Pullalah 1999; Riahi et al. 2003; Faigo´n-Soverna et al. 2003; Moço and Mariath 2003; Zulkarnain 2005; Galati et al. 2006; Rezanejad 2007; Rodriguez-Pontes 2008; Riahi and Zarre 2009; Salinas-Gamboa et al. 2016) and a few embryological studies in *Onobrychis* (Chehregani et al. 2011; Ghassempour and Majd 2012) have provided useful characters in embryogenesis and classification. Therefore, in this study we investigate detailed embryological processes in *O. persica* using bright field, polarizing and fluorescence microscopy and compare them with some spices in this genus. These valuable characters can be used to evaluate the genera of this tribe and investigate their position.

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Materials and methods

The voucher specimen is deposited at the Bu–Ali Sina University Herbarium (BASUH 1098) and labeled as follows: Iran, Hamedan, Kabudar Ahang, Subashy village, alt. 2236 m.

Light microscopy (LM)

Tiny buds, flowers and fruits in different developmental stages, at least 30 samples for each developmental stage from the same population, were fixed in FAA70, stored in 70% ethanol, embedded in paraffin, and sectioned at 4–6 µm with Micro DC 4055 microtome. Staining was carried out with the periodic acid Schiff (PAS) and Meyer's hematoxylin techniques. Several sections were viewed under a Zeiss Axiostar Plus bright field microscope for each embryological developmental stage.

The sections of seeds were stained with different histochemical techniques: toluidine blue O (TB-O) 0.5% aqueous solution, pH 3.0 (Merck, Darmstadt, Germany) was used in order to identify phenols and acidic polysaccharides (O'Brien et al. 1964; Gordon and McCandless 1973); periodic acid–Schiff (PAS) to identify neutral polysaccharides (Gahan 1984); Lugol to identify starch grains (Johansen 1940); and Coomassie Brilliant Blue (CBB) 0.4% in Clarke's solution (Serva, Heidelberg, Germany) to identify proteins (Fisher 1968; Gahan 1984). Sudan Black B (SB–B) staining was used to identify lipids (Johansen 1940). Sections were analyzed with a Canon G_{11} digital camera (Tokyo, Japan) attached to a Zeiss Axiostar Plus microscope (Medac, Wedel, Germany). Polarized light was also used for detection of birefringent molecules.

Fluorescence microscopy (FM)

The sliced specimens, without staining or after staining, were analyzed using an ultraviolet (UV) light-emitting diode (wavelength of 405 nm) in the epifluorescent microscope (Bell, Monza, Italy) equipped with the Image Capture Q Capture Pro 5.1 Software (Qimaging Corporation, Austin, TX, USA). Fluorochromes (aniline blue) and autofluorescence investigation were used in an epifluorescence system equipped with a UV filter (WU: 330–385 nm), a dichroic mirror (400 nm) and a barrier filter (420 nm).

Pollen tubes were also examined using fluorescence microscopy. The samples were softened in 1.0 M NaOH for 1 h, rinsed in distilled water for 0.5 h, and stained in 0.1% solution of aniline blue in 0.1 M K_2HPO_4 for 4 h. Pollen tubes were viewed with UV epifluorescence using a BP 355–425 excitation filter and a LP 460 barrier filter.

Results

Male gametophyte

Anther development starts when the flowers are very small. They are ovoid shaped and tetrasporangiated (Figures 32, 33). The anther wall development follows the dicotyledonous type, consists of four layers: epidermis, endothecium, the middle layer and a secretory tapetum (Figure 32). Microspore mother cells (MC) are surrounded by a callose coat. They contain a big nucleus at the center (Figures 26, 27). The tapetal cells are uni-nucleate (Figure 29). Cytokinesis is simultaneous in MC, after meiosis and the arrangement of microspores is tetrahedral and tetragonal. They are surrounded by a callose coat (Figure 28).

Microspores undergo unequal mitotic division and produce a small generative and a large vegetative cell (Figure 30). Ellipsoidal tricolpate pollen grains are two-celled at the time of shedding (Figure 30). In this time tapetum degenerates completely and only endothecium and epidermis are visible. Fibrous thickenings are developed in the endothecium when shed (Figure 31). Growing and branching of pollen tube in the pistil and style is visible using fluorescence microscopy. Aniline blue staining showed pollen tube by fluorescence microscopy (Figures 35, 36, 37).

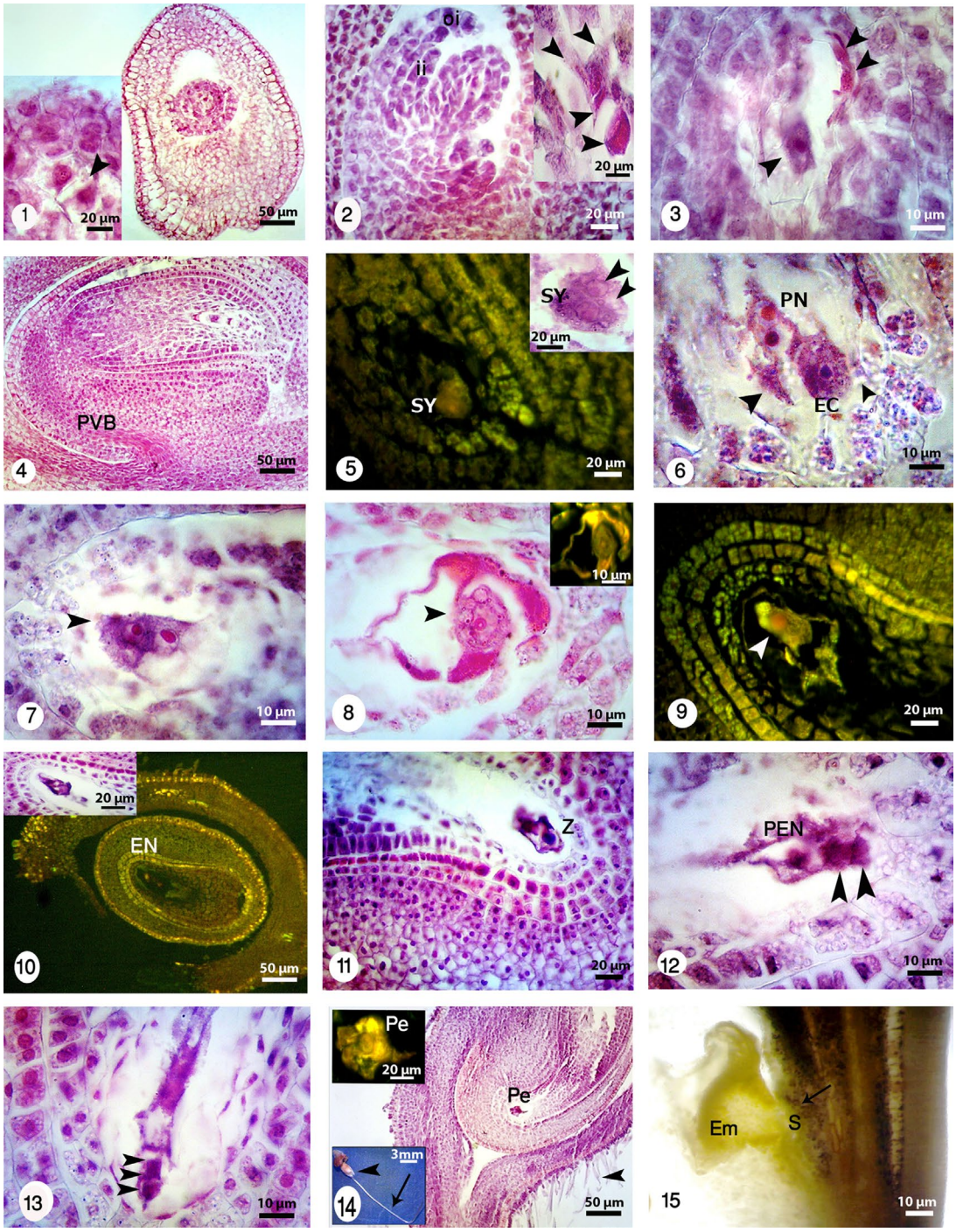
Female gametophyte

Megasporocytes undergo successive meiosis to forms unequal dyad cells (Figure 1) and then four megaspores in a T-shaped tetrad (Figure 2). The functional megaspore, which is located in the chalazal position, undergoes three successive stages of mitotic divisions and develops into an eight-nucleate embryo sac. The pattern of the embryo sac development follows a *Polygonum* type: the egg cell and two synergids that formed egg apparatus, the central cell that is the largest cell and three antipodal (Figure 4).

The young ovule is hemianatropous but the mature one is anatropous, crassinucellar (Figure 4) and bitegmic; integuments form a zigzag micropyle. A vascular bundle (procambium) begins to differentiate in the funiculus (Figure 4).

After cellularization of the embryo sac two polar nuclei are close to the egg apparatus (Figures 6, 7). The polar nuclei remain separated from each other until fertilization. The multilayered coenocytic nucellus becomes partly cellular (Figures 5, 10). As the embryo sac enlarges, the cone-shaped embryo sac changes to a U-shaped one and the nucellar cells begin to degenerate (Figures 4, 10, 14). After fertilization, the inner layer of the inner integument forms endothelium with cube-shaped cells (Figures 10, 11).

The inner integument consists of two cell layers, while the outer one is composed of four to five layers at its thinner part (Figures 2, 4). In *O. persica*, the ovary has



Figures 1-15. Longitudinal sections showing embryo sac development under bright field and fluorescence microscopes and different magnifications. 1: Young ovary containing a young ovule (right) showing dyad stage (right and left) (*black arrowhead*). 2: Two different magnifications of a T-shaped tetrad (*black arrowheads*). The outer integument (oi) grows faster than inner one (ii). 3: Three nuclei of four-nucleated embryo sac (*black arrowheads*). 4: Anatropous, crassinucellar and bitegmic ovules with nucellus and provascular bundle (PVB). The cone-shaped embryo sac with egg apparatus, two polar nuclei, antipodal cells and zigzag micropyle. 5: One of the synergids (SY) and oospher with its polarity are visible under bright field (*black arrowheads*) and fluorescence microscopes. 6: Synergids (*black arrowheads*), egg cell (EC), two polar nuclei before fusion and degeneration of micropylar end is visible. 7: Two polar nuclei and their finger-like tubers. 8: Egg apparatus and secondary nucleus (*black arrowhead*) formation after fusion of polar nuclei under bright field (left) and fluorescence (right) microscopes. 9: Mature embryo sac with differentiated egg (*white arrowhead*) and its polarity. 10: Sagittal section through the embryo sac under bright field and fluorescence microscopes with two different magnifications. Nucellar cells are degraded in the middle of embryo sac but persistent adjacent to the chalazal areae. Endothelium layer (En) is distinguishable at the inner integument. 11: Longitudinal section of zygote (Z) and synergids. 12: The primary endosperm nucleus (PEN), apical and basal cells (*black arrowheads*). 13: Basal cell and 2-celled proembryo (*black arrowheads*). 14: The U-shaped embryo sac and proembryo (Pe) under bright field (right) and fluorescence microscopes (left). The ovary with trichomes (in different magnifications; *black arrowheads*), long style (*black arrow*). 15: Globular embryo (Em) with young suspensor (S) surrounded by coenocytic endosperm. Staining with lugol indicates accumulation of starch grains at the base of embryo (*black arrow*).

long peduncle and trichomas (Figure 14). The young ovary has one ovule and the mature legume contained only one seed, but rarely a two-seed legume is possible (Figure 18). In zygote stage, the nucleus of the zygote is not easy to distinguish due to density of cytoplasmic materials (Figure 11).

The zygote undergoes a transverse division to form the apical and basal cells (Figure 12). A small proembryo forms after several divisions (Figure 14). It differentiates into a globular embryo and a short suspensor (Figure 15). The coenocytic endosperm in this stage is still around the embryo. Staining with lugol indicates starch grain accumulation at the base of embryo (Figure 15).

At the heart stage, the suspensor is composed of one cell column. At cotyledon stage the suspensor shows signs of degeneration (Figure 25).

During embryogenesis the inner integument begins to degenerate (Figure 10) and the outer integument produces several distinct cell layers and establishes the "typical" seed coat structure. The outer integument cells elongate and differentiate to the macrosclereid cells, characteristic for the testa of Fabaceae, with phenolic compounds that give a green color by toluidine blue (Figure 21). The macrosclereids are large with a well-developed light line in the outer wall and thick cuticle (Figures 21, 24).

In seed coat a sub-epidermal layer of cells is differentiated into osteosclereid layer with large intercellular spaces (Figure 21). Beneath these are thin-walled parenchymatous cell layers. Parenchyma cells are the innermost part of the seed coat with the inner layer in direct contact with the endosperm. This layer of endosperm (aleuronic layer) remained attached to the parenchyma layer (Figures 20, 22, 23). Lipid reserves were determined in endosperm, aleuronic layer and cotyledons; by staining with Sudan black B which reacts positively for lipids (Figure 20).

Starch grains are absent in macrosclereids but there are high accumulation of these grains in osteosclereids, and parenchyma cells by staining with lugol (Figures 21, 22, 24, 25). Cotyledons accumulate starch (Figure 25).

At the hill pole, the Faboideae seed characteristic structure develops, with double palisade layer, subhilar parenchyma, and tracheid bar (Figures 19, 22, 23). This strip of large, pitted and lignified tracheids is commonly found in legumes.

Thus, we can show several compound and different layers with different staining beside hematoxylin techniques.

Discussion

Comparative embryology has been an important factor in revealing taxonomic relationships of taxa at higher levels (Johri et al. 1992). Comparative embryology of our present, previous results on *Onobrychis* and literature for Fabaceae are summarized in Table 1. *O. persica* shares a number of embryological characters with other species of *Onobrychis* and Fabaceae (Table 1): tetrasporangiate anthers, fibrous endothecium, secretory tapetum, two-celled mature pollens, tricolpate pollen, *Polygonum* type embryo sac, crassinucellate, bitegmic and anatropous ovule, chalazal functional megaspore and number of layers in inner integument.

In *O. persica* the mature embryo-sac has a *Polygonum* type of development that is common in this family (Riahi et al. 2003; Moço and Mariath 2003; Galati et al. 2006; Rezanejad 2007; Rodriguez-Pontes 2008; Bakar Büyükkartal 2009). This type is the most common in angiosperms and is considered as the primitive embryological character (Liu et al. 2003).

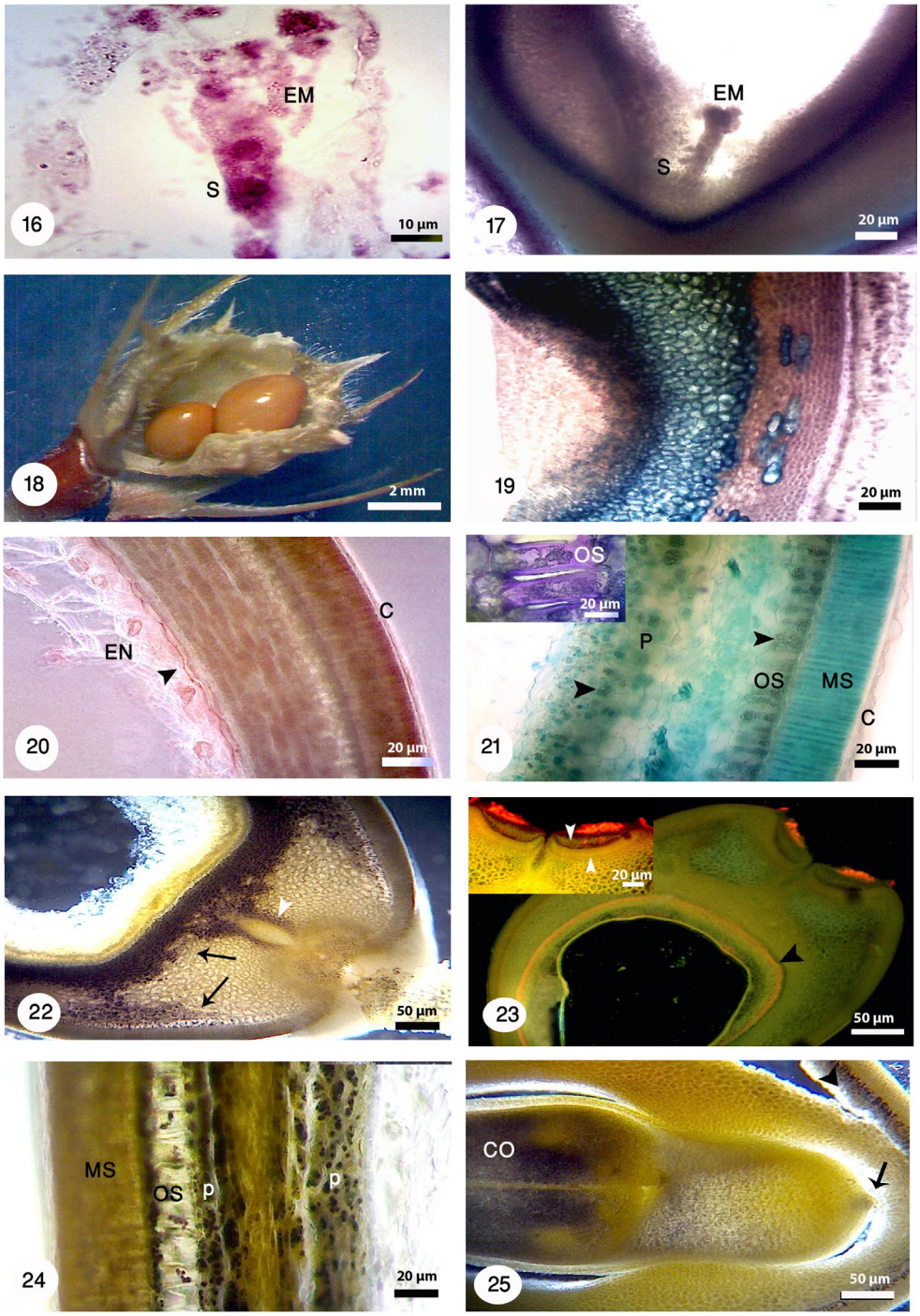
The ovules are crassinucellate in Fabaceae (Faigo ´n-Soverna et al. 2003; Riahi et al. 2003; Moço and Mariath 2003; Galati et al. 2006; Rezanejad 2006), which were also found in *O. persica.* Asymmetrical growth at the funicular region is the reason for this anatropous curvature in *O. persica*, like other legumes (Bouman and Boesewinkel 1991; Galati et al. 2006; Rezanejad 2006; Riahi and Zarre 2009) and our study indicates that the young ovule is hemianatropous and then it change to anatropous one.

The present study indicates that the chalazal megaspore is functional like other species of *Onobrychis* that we studied before (Chehregani et al. 2011) and *Vigna unguiculata* L. Walp. (Salinas-Gamboa et al. 2016). But variations in the position of the functional megaspore have been reported in other Fabaceae, e.g. *Trifolium repens* (Martin 1914) and *Vicia faba* L. (Mitchell 1975) with an epichalazal position in *Milletia ovalifolia* Kurz (Pal 1960), *Trifolium hybridum* (Kazimierski and Kazimierski 1979) and in some Australian species of the tribe Mirbelieae (Cameron and Prakash 1994), where it may be in a chalazal, micropylar or epichalazal position.

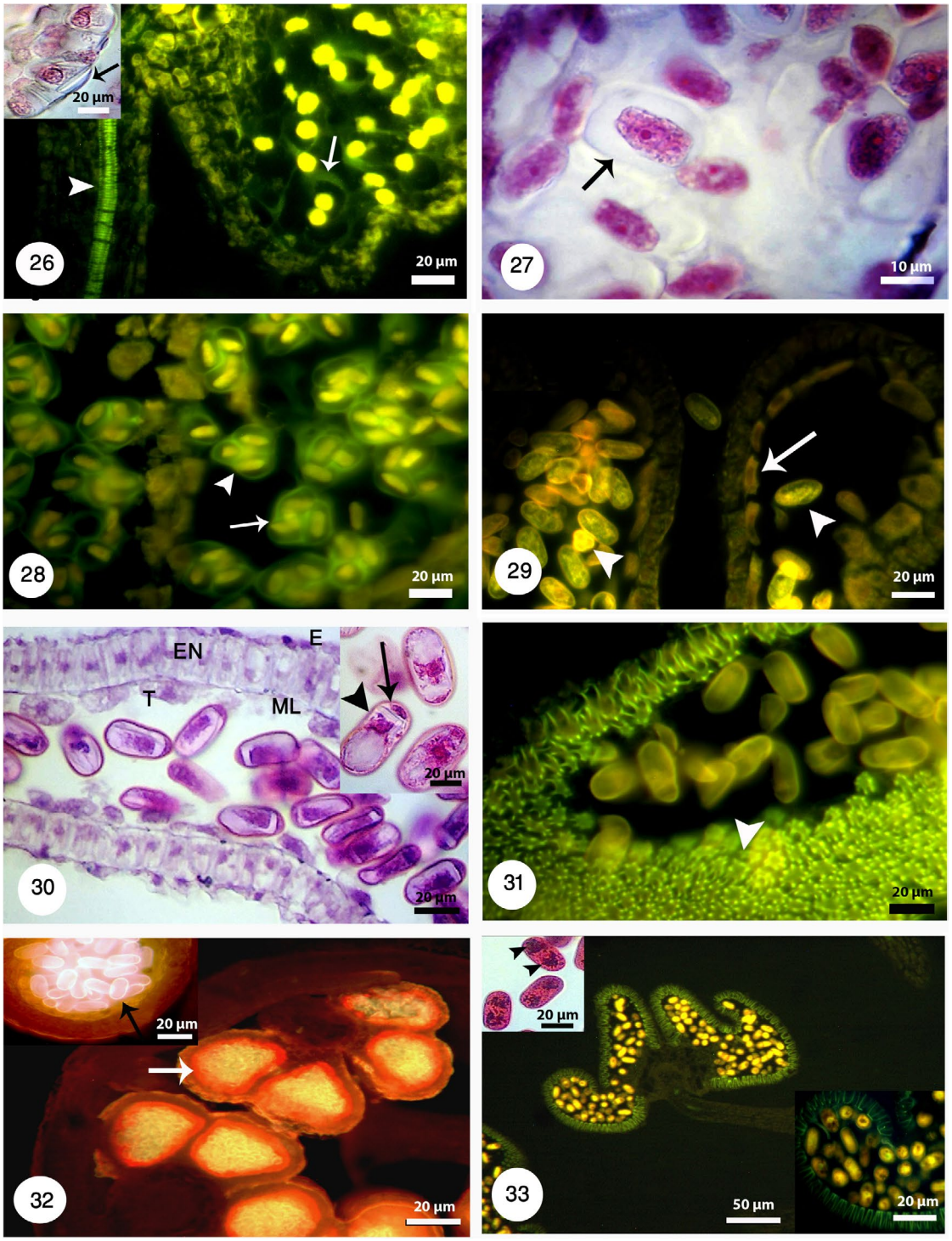
In *O. persica*, the inner integument, as our previous studies on *Onobrychis* (Chehregani and Tanaomi 2010; Chehregani et al. 2011) and most Fabaceae, consists of two cell layers (Smith 1956; Dnyansagar 1957; Hindmarsh 1964; Deshpande and Bhasin 1976; Rembert Junior 1977; Ashrafunnisa and Pullaiah 1994, 1999; Moço and Mariath 2003; Riahi and Zarre 2009; Vardar 2013). However, in *Glycine javanica* L., *Tephrosia*, *Clitoria ternata* L., *Pongamia glabra* Vent. (Anantaswamy 1951), *Teramnus labialis* (L.f.) Spreng. (Anantaswamy 1953), *Psophocarpus tetragonolobus* (L.) DC. (Lim and Prakash 1994) and also in the species of *Cassia* (Pantulu 1945) it is formed by more than two layers.

Although there is the same developmental pattern in *O. persica*, other species of *Onobrychis*, and Fabaceae, comparative study showed several significant embryological features of *O. persica* that differ from those (Table 1). These will be discussed in detail below.

The number of middle layer differs between different species. There is one middle layer, dicotyledonous type,

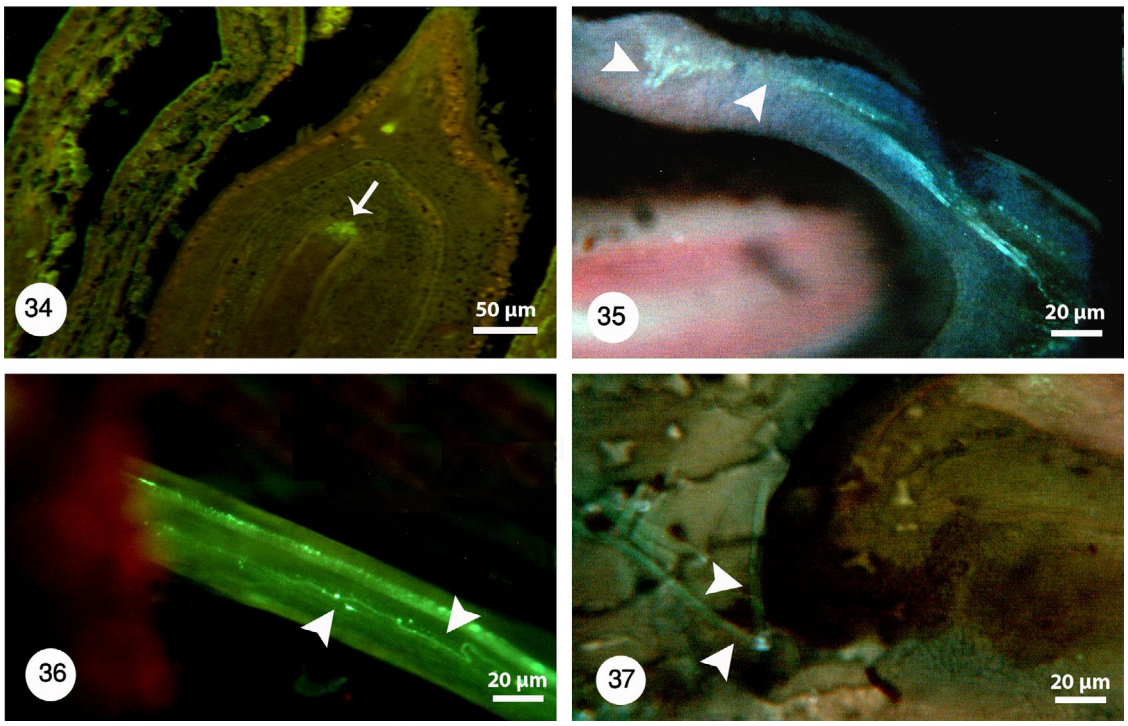


Figures 16-25. Different developmental stages of seed and embryo in *Onobrychis persica* under bright field and fluorescence microscopes with different magnifications. 16: Early heart-shaped embryo (EM) with suspensor (S) consisting of one column of cells. 17: The heart-shaped embryo (EM) and suspensor (S). 18: *Onobrychis* mature legume contained just one seed but the legumes with two seeds were also observed. 19: Longitudinal section of seed stained with methylene blue shows the distinguishable layers in seed. 20: Transverse section of seed stained with Sudan black B and polarizing microscopy shows lipid reserves in cuticle layer (C) and endosperm (EN) specially in aleurone layer (*black arrowhead*). 21: Staining of seed coat and endosperm with toluidine blue indicates starch grains (*black arrowheads*); positive reaction for phenolic compounds gives a green color. Starch grains are absent in macrosclereids (MS) but there are high accumulation of starch grains in osteosclereids (OS) and parenchyma cells (P). Cuticle layer (C) is thick. 22: Staining with lugol indicates starch grains (*black arrows*). Storage of starch grains during embryogenesis is visible in the seeds. The tracheid bar is distinguishable (*white arrowhead*). 23: At the hilar pole, double palisade layer (*white arrowheads*), subhilar parenchyma, and tracheid bar is distinguishable. The aleurone layer is also noticeable (*black arrowhead*) in the larger figure. 24: A part of testa showing macrosclereids (MS), osteosclereids (OS) and parenchyma cells (P), and accumulation of starch grains. 25: Cotyledon (CO) stage which accumulate starch. In this stage suspensor shows signs of degeneration (*black arrow*). Note: In Figures 19–25 seed sections were stained with different staining techniques and different microscopes were used (bright field, polarizing and fluorescence).



Figures 26-33. Anther structure under bright field and fluorescence microscopes with different magnifications. 26: Microspore mother cells surrounding by a callose coat (*white arrow*) in a young anther with undifferentiated wall (*black arrow*). The vascular bundles are distinguishable (*white arrowhead*). 27: Microspore mother cells are surrounded by a callose coat (*black arrow*). A big nucleus is visible at the center. 28: The arrangement of tetrads inside the callose walls is tetrahedral (*white arrow*) and tetragonal (*white arrowhead*). 29: Released microspores in polar and equatorial view (*white arrowheads*). The tapetal cells are uni-nucleate (*white arrow*). 30: Microspores undergoes mitotic division and produces a vegetative cell (*black arrowhead*) and a generative cell (*black arrow*). The anther wall consists of four layers: epidermis (E), endothecium (EN), the middle layer (ML) and a secretory tapetum (T). 31: Fibrous thickenings are developed in the endothecium (*white arrowhead*). 32: The anther is tetrasporangiate with thick exine *schahuensis* are T-shaped microspores (*black arrow*). The anther wall layers are distinguishable with fluorescence microscope (*white arrow*). 33: Two-celled mature pollen with generative and vegetative nuclei (*black arrowheads*) in a mature anther.

or sometimes two middle layers, basic type, in Fabaceae. In *O. persica*, there is just one middle layer in the anther wall so the anther wall formation is of dicotyledonous type.



Figures 34-37. 34: Degeneration of micropylar end during fertilization (*white arrow*). Pollen tube growth with fluorescence microscopy and aniline blue staining: 35: Pollen tubes stained with aniline blue (*white arrowheads*) shows growing and branching on the tube in the space of between integuments. 36: Longitudinal section of the pistil showing pollen tube growth (*white arrowheads*) through the short open stylar canal that leads to an ovule. 37: Pollen tube growing through the micropyle (*white arrowheads*).

The tapetal secretory cells are regularly uni-nucleate in *O. persica*, *Onobrychis altissima* Grossh., *Onobrychis melanotricha* Bornm., *Onobrychis andalanica* Bornm. and most of Fabaceae. But in *Onobrychis schahuensis* Bornm. these cells have one or two nuclei (Table 1). Our results indicate that the arrangement of microspores in *Onobrychis* species and often in Fabaceae is tetrahedral and tetragonal with simultaneous cytokinesis (Table 1).

The tetrads of megaspores in *O. persica*, most legumes and our previous study on *Onobrychis* except for *O. schahuensis* are T-shaped (Table 1) (Chehregani and Majd 1992; Faigo´n-Soverna et al. 2003; Riahi et al. 2003; Chehregani et al. 2011). But the tetrad shape in Fabaceae can be different (Moço and Mariath 2003; Galati et al. 2006; Rodriguez-Pontes 2008). Rembert Junior (1966, 1967, 1969a, 1969b, 1971) used the marked variability in the great megaspore tetrads to form phylogenetic hypotheses in Leguminosae.

In the chalazal end of embryo sac the antipodal cells are relatively persistent (Table 1). Suspensor morphology is variable in the Fabaceae (Lersten 1983). In *Phaseolus* it attains a club-like shape and contains about 200 cells (Cionini 1987), while the suspensors of *Senna corymbosa* (Rodriguez-Pontes 2007) and the subtribe Cassiinae (De-Paula and Oliveira 2012) are poorly developed. In the present study the suspensor is composed of one column of cells with its maximal length at the heart-shaped stage of the embryo, and begins to degenerate at the cotyledon stage, like in *Astragalus cemerinus* and *A. ruscifolius* (Riahi and Zarre 2009). Our results showed that the mature legume contains one seed, but every so often the legume contains two seeds. Typically *Onobrychis* is identified and separated from the other groups because of its one seed legume (Mabberley 1990; Lock 2005) and there are numerous seeds in different Fabacean species (Riahi and Zarre 2009; Chehregani et al. 2011). It is a remarkable finding that we have observed two seeds in legume of *O. persica.* The outer integument with several cell layers is common in Fabaceae (Galati et al. 2006; Rodriguez-Pontes 2008; Vardar 2013). In *O. persica*, the outer integument has four or five layers, but in *Astragalus cemerinus*, *A. ruscifolius* and *Swainsona formosa* the outer integument is two or three cell layers thick (Zulkarnain 2005; Riahi and Zarre 2009).

Differentiation of the integuments first occurs in the inner integument with the formation of the endothelium at the early stages of ovule development. The endothelium is a new limiting layer of the embryo sac in many ovules (Bouman 1984). In *Phaseolus coccineus* L. the endothelium is present until the cotyledon stage (Yeung and Clutter 1978), but in *O. persica* it is distinguishable until heart embryo stage. In *Vicia faba* the degradation of the inner integument and additional maternal tissues occur during the initial development of the embryo (Johansson and Walles 1994). Our results indicate that the outer integument begins to take on the morphology of the mature testa at early stages of development.

In *O. persica* the development of the large macrosclereid with a well-developed light line and osteosclereid was similar to the development pattern observed

Table 1. Embryological comparison among five species of *Onobrychis* and Fabaceae.

Features	*O. persica*	*O. altissima*	*O. melanotricha*	*O. andalanica*	*O. schahuensis*	Fabaceae	References
Anther wall formation	Dicotyledonous	Dicotyledonous	Dicotyledonous	Dicotyledonous	Dicotyledonous	Dicotyledonous and basic	Teixeira et al. 2002; Liu et al. 2003; Rezanejad 2007
Middle layer	1	1	1	1	1	variable	Teixeira et al. 2002; Liu et al. 2003; Rezanejad 2007
Tapetum	Secretory	Secretory	Secretory	Secretory	Secretory	Often secretory	Suzuki et al. 2001; Wilson 2001; Teixeira et al. 2002; Liu et al. 2003; Feng et al. 2006; Rezanejad 2007; Chehregani et al. 2008; Hashemi and Rezanejad 2013
Number of tapetal cell nuclei	1	1	1	1	1-2	Often 1	Suzuki et al. 2001; Teixeira et al. 2002; Liu et al. 2003; Galati et al. 2006; Rezanejad 2007
Cytokinesis in meiosis	Simultaneous and successive	Simultaneous and successive	Simultaneous and successive	Simultaneous and successive	Simultaneous and successive	Often successive	Suzuki et al. 2001; Wilson 2001; Teixeira et al. 2002; Liu et al. 2003; Feng et al. 2006; Rezanejad 2007; Chehregani et al. 2008; Hashemi and Rezanejad 2013
Microspore tetrads	Tetragonal and tetrahedral	Tetragonal and tetrahedral	Tetragonal and tetrahedral	Tetragonal and tetrahedral	Tetragonal and tetrahedral	Often tetrahedral	Suzuki et al. 2001; Wilson 2001; Teixeira et al. 2002; Liu et al. 2003; Galati et al. 2006; Rezanejad 2007
Type of aperture	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Often tricolpate	Pavlova and Manova 2000; Liu et al. 2003
Mature pollen	Two-celled	Two celled	Two celled	Two celled	Two celled	Variable	Liu et al. 2003; Galati et al. 2006
Ovule number	1–2	1	1	1	1–2	Variable	Hashemi and Rezanejad 2013
Curvature of ovules	Hemianatropous and anatropous	Anatropous	Anatropous	Anatropous	Anatropous	Hemianatropous and anatropous	Moço and Mariath 2003; Faigo´n-Soverna et al. 2003; Galati et al. 2006; Rezanejad 2006; Riahi and Zarre 2009; Rodriguez-Pontes 2007
Nucellus type	Crassinucellate	Crassinucellate	Crassinucellate	Crassinucellate	Crassinucellate	Crassinucellate	Moco and Mariath 2003; Faigo´n-Soverna et al. 2003; Galati et al. 2006; Rezanejad 2006; Riahi and Zarre 2009; Rodriguez-Pontes 2007
Number of integument	Bitegmic	Bitegmic	Bitegmic	Bitegmic	Bitegmic	Bitegmic	Moco and Mariath 2003; Faigo´n-Soverna et al. 2003; Galati et al. 2006; Rezanejad 2006; Riahi and Zarre 2009; Rodriguez-Pontes 2007
Number of layers in inner integument	2	2	2	2	2	Often 2	Ashrafunnisa and Pullaiah 1999; Galati et al. 2006; Rodriguez-Pontes 2008
Number of layers in outer integument	4–5	4–5	3	3	4–5	Several layers	Galati et al. 2006; Rodriguez-Pontes 2008
Megaspore tetrads	T-shaped	T-shaped	T-shaped	T-shaped	Linear shaped	Linear shaped and T-shaped	Moco and Mariath 2003; Galati et al. 2006; Rodriguez-Pontes 2008
Embryo sac	Polygonum	Polygonum	Polygonum	Polygonum	Polygonum	Often polygonum	Faigo´n-Soverna et al. 2003; Moco and Mariath 2004; Galati et al. 2006; Rezanejad 2006; Rodriguez-Pontes 2007
Antipodal cells	Persistent	Persistent	Persistent	Persistent	Persistent	Variable	Johansson and Walles 1994
Seed number	1–2	1	1	1	1	Variable	Riahi and Zarre 2009; Chehregani et al. 2011
Amyloplast accumulation in embryo sac	Too much	Too much	Too much	Much	Few	Variable	Chehregani et al. 2011
Ovary peduncle and hair	+	+	+	+	–	Variable	Chehregani et al. 2011
Selected references	Present study	Chehregani et al. 2011	Chehregani et al. 2011	Chehregani et al. 2011	Chehregani et al. 2011	*	

in *Pisum sativum* L. (Harris 1984), *Glycine max* Merr. (Harris 1987), *Erythrina lysistemon* Hutch. (Manning and Van Staden 1985), and *Trifolium pratense* L. (Algan and Bakar Büyükkartal 2000). The macrosclereid layer is responsible for water impermeability in Fabaceae seeds (Riahi et al. 2003; Rodriguez-Pontes 2007, 2008; Muneratto and Souza 2013; Varela and Albornoz 2013). A layer of parenchyma tissue is present in all studied Fabaceae (Riahi et al. 2003; Rodriguez-Pontes 2007, 2008; Muneratto and Souza 2013; Varela and Albornoz 2013).

The storage of lipid and starch grains during embryogenesis can be illustrated with different staining techniques. In some species, starch is ephemeral in the seeds, and is a temporary form of reserve. In *Glycine max* these grains were not observed in endosperm (Chamberlin et al. 1994). Morphological characteristics of seeds are often used in taxonomical classifications in Fabaceae (Riahi and Zarre 2009; De-Paula and Oliveira 2012; Güneş 2013). Also with different staining we can recognize valuable characters in seeds that can be very useful in taxonomical analysis, such as the number of layers, presence or absence of some layers, thickness of layers and many different compounds in each layer. We can also study different developmental stages of each seed layers with this specific staining.

Based the embryological data (Table 1) we can conclude that *O. persica* has the most similarity with *O. altissima*. These two species are placed in *O.* section and have a chromosome number of $x = 7$ (Hesamzadeh Hejazi and Ziaei Nasab 2010; Ranjbar et al. 2010; Arslan et al. 2012). These embryological findings confirm the position of the species based on morphological and karyological analysis. Our embryological findings indicated that *O. persica* is different from *O. melanotricha* and *O. andalanica* (from *Heliobrychis* section) in terms of seed number and number of layers in outer integument. According to Table 1, embryological characters of *O. persica* is most different to *O. schahuensis*, from *Hymenobrychis* section: in terms of number of tapetal cell nuclei, curvature of ovules, megaspore tetrads, seed number, amyloplast accumulation in embryo sac and ovary peduncle, and hair. In conclusion, our embryological findings are in agreement with the taxonomical positions of these species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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