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Ovule Ontogenesis and Megagametophyte Development in *Onobrychis sintenissii* Bormn. (Leguminosae-Papilionoideae)

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Abstract

There have been few reports available on wild legume embryology and fewer studies have addressed the formation of *Onobrychis sintenissii* Bormn. The purpose of this research is to study the formation stages of a flower, microporogenesis and femel gametophyte development. In this research, the development of the ovule and the formation of the gametophyte on the coloured sections of *O. sintenissii* were studied using a light microscope. The primordial shows a tetra-zonate structural organization. The two integuments are initiated by perclinal divisions in the dermal layer (zone I), around the base of the archesporium. Subsequently, cells derived from the subdermal layer (zone II) start to push the dermal cells, so that they shift toward the micropylar region; the outer integument grows in a faster rate compared to the inner one. In ovule primordium, a laterally positioned subdermal cell (zone III & zone IV) grows and differentiates as an archesporial cell, containing a prominent nucleus and a dense cytoplasm, which differentiates directly into the megaspore mother cell, undergoes meiosis, and originates a linear tetrad of megaspores. The young ovule is hemi anatropous but the mature one is anatropous, crassinucellar and bitegmic integuments. The mature embryo sac mother cell is elongated, possessing a conspicuous central nucleus and a *polygonum* type of development. The embryological characters of *O.sintenissii* are compared with those of other texa within the leguminosae, such as the tetra-zonate ovule primordium, anatropous ovule type, dermal origin of the integuments, asymmetrical initiation of the outer integument, linear shaped tetrad with the presence of one functional megaspore, and having two young ovules and forming of two embryoes.

Keywords: Embryo sac, embryology, leguminosae, megaspore, *Onobrychis*, ovule.

INTRODUCTION

There are few embryological reports on wild legumes. The ovule ontogenesis, details of the megagametophyte and embryo sac mother cell differentiation has scarcely been studied in leguminosae. The ovules are anatropous, hemi anatropous, amphitropous or campylotropous [1](Bocquet and Bersier,1960) and always bitegmic have been described [2](Lersten, 2004) and a zigzag micropyle[3] (Prakash, 1987). Embryologically, the family is characterized by the presence of a monosporic, seldom bisporic [4,5](Rembert, 1966, 1967), megagametogenesis. This begins with a multicellular archesporium [3](Prakash, 1987), a cell of which develops into the megaspore mother cell (MMC), and after meiosis generally becomes a linear or T-shaped megaspore tetrad. Morphological characters of the ovules and details of megasporogenesis can be used in systematic studies. For defining the circumscription of the genus. The purpose of this study is to investigate megasporogenesis, embryo sac mother cell maturation and formation of the integument, in *Onobrychis sintenissii*; endemic in Iran.

MATERIALS AND METHODS

To study megasporogenesis, megagametogenesis ovule ontogeny, flowers at anthesis and flower buds at different stages of development were analyzed. They were fixed in FFA (formalin: glacial acetic acid: ethanol 5:5:90 parts with 70% ethanol). The material was embedded in paraffin, cut into 8-14 m sections and treated according to classical paraffin methods. The sections were stained with hematoxylin and eosin. The microphotos were made with Nikon ECLIPSE (DXM 1200). All the sections were studied under a light microscope.

RESULT

Flower Primordium Development

Microscopic observation of transverse section of the bud (0.4-1mm) indicated that the stem cell divides and forms the primordia in *O. sintenissii*. The primordium in the flower organ is usually formed by priclinal division in the outer layer of the flower peak and anticlinal and perclinal or oblique division in the second and and so on layer (Fig.1A). The development of different section of the flower in *O. sintenissii* begins with the formation of sepal primordial (Fig.1B). And then cell divisions and differentiation of stamen primordial (Figs.1C-1D)and pistil (Figs.1E-1F) follows. Petal primordial is formed at the last stages of the flower development. Primordium cells of the flower are extremely color-absorbent, which is an indication of their biosynthesis activities and high rate of cell divisions.

Ovule Development

According to the pattern observed for cell divisions, before ovule ontogeny begins, as we see in a transverse section of the ovary, the ovule premordium has a tetrazonate structure. The dermal layer (zone I) undergoes only anticlinal divisions. The subdermal layer also follows the same pattern (zone II). Although some oblique or periclinal divisions are observed in one or some cells in this layer. Cellular divisions in the tissue underlying the subdermal layer (zone III & zone IV) follow no regular pattern (Figs. 2A-2B). From the placental tissue of the ovary, the ovule premordium arises in 2 rows like small protuberances and curve towards the stylar end, and the early rudiment of the integument appears from the epidermal cells(Fig. 2C). In ovule premordium, a laterally positioned subdermal cell grows and differentiates as an archesporial cell, containing dense cytoplasm and a prominent nucleus(Fig. 2D). Once the margins of the integument reach the top of the nucellus, they project beyond it and originate the micropyle. An archesporial cell is divided into some parietal cells while a sporogenous cell is enlarged, becoming the megaspore mother cell (MMC) (Fig. 3A). The mother cell is in the nucellus and partly covered with the integuments which are developing, and it has a prominent nucleus. The nucellus is quite reduced, and crassinucellate (type I nucellus, see Nikiticheva, 2002: Fig. 3D), and ending in the chalaza is considered to be the raphe (Fig. 3B). A vascular bundle (procambium) begins to differentiate in the funiculus, which crosses the funiculus and ends at the base of the nucellus (chalaza). The first meiotic division occurs in the MMC and forms a dyad (Fig. 3B) and then after sounds like the chalazal member of the dyad undergoes the 2nd meiotic division. This is while no divisions occur in the micropylar one resulting

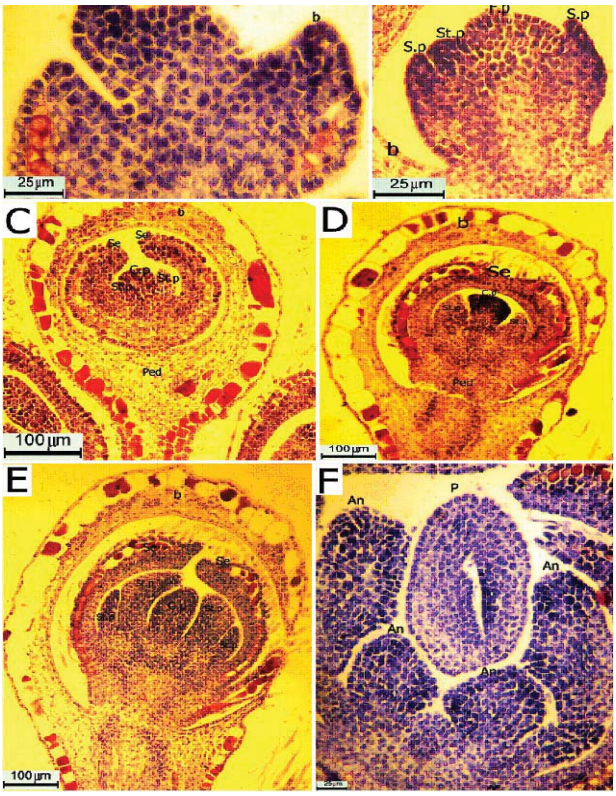

Figure 1. A transverse section of the bud (0.4-1mm), showing flower primordium in successive stages of development.
(A) The flower primordium develops in the small buds. (B) Forming of bract and development of sepal and stamen primordium. (C) Development of sepal and stamen primordium. (D) Forming of two stamen primordial and development of pistil. (E) Development two peripheral stamen primordium and central pistil primordial. (F) Anther at the early microspore mother cells stage .note the pistil development. An:Anter; b:bract; C.p: Carpel F.p: flower primordial; Ovul: Ovule P:pistil; Ped:Pedicale; Se: Sepal; Primordial; S.p: Sepal primordial; St.p: Stamen Primordial; Styl:Style;

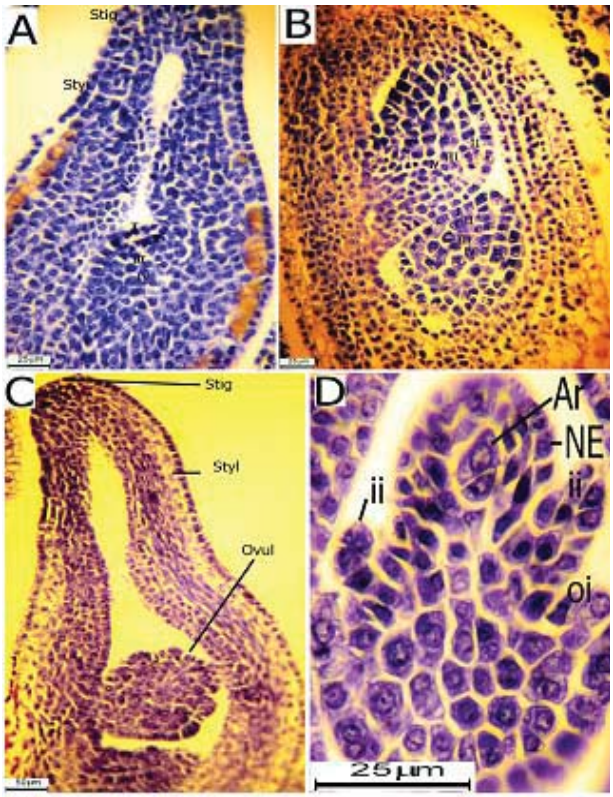

Figure 2. Transversesection of the ovary showing ovules in successive stage of development.
Form (A) to (B) the limits among the zones I (dermal layer), II (subdermal) and III-Iv are represented by a thicker line. (C) ovule initiation is basipetal and start with miotatic activity meristemic regions organizerd in four layers; (before emergence of the nucelli). (D) The initial archesporial cell is distinguished from the other sub-dermal cell;(ovule at the beginning of integument development). Ar:Arcegon; ii: Inner Integument; NE: Nucellar Epidermis; Oi; Outer Integument; Stig: Stigma;

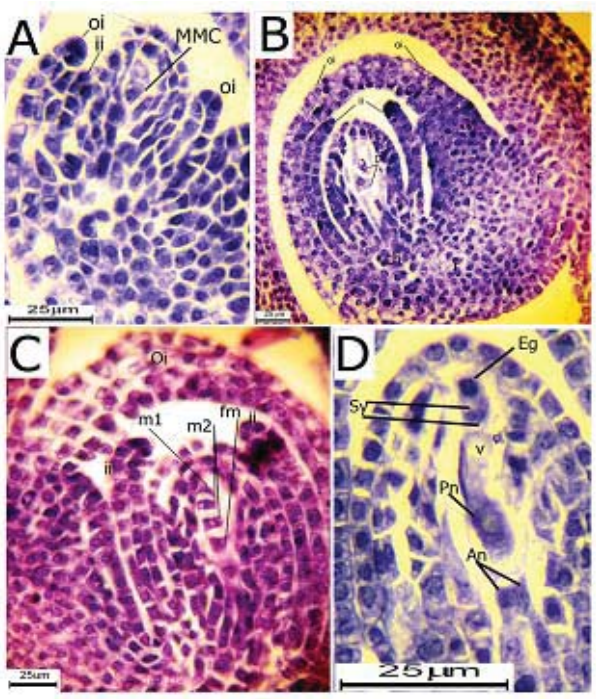

Figure 3. Developmental stages of an ovule
A: Megaspore mother cell formation and approximity of outer layers to one another. B: The first Meiosis of megagametosit and formation of a dyad. C: Ovule in tetrad stage. D:Arrangement of the seven cells in the gametophyte. ch:Chalaza; D:Dyad; Eg:Egg; f: Fetal; fm:Functional Megaspore; ii: Inner Integument; m1: Micropylar Cell; m2:Intermediate Megaspore ; MMC: Megaspores Mother Cell; Oi:Outer Integument; Pn: Polar Nucleus; Sy: Synergids; V:Vacuole;

in a linear triad of cells (Fig. 3C). It is important to note that chalazal member becomes the functional megaspore whilst the rest degenerate. The mature ovule is anatropous. The inner and outer integuments consist of 2 layers except in the micropylar region where they may become multi-layered micropyle. However, because of the curvature of the ovule, the funicular side of the outer integument is far less developed, being practically reduced to the micropylar area, and is thus mostly multi-layered (Fig. 3B). The functional megaspore undergoes the successive mitotic divisions to produce an 8-nucleate mega gametophyte, Initially seven-celled/eight-nucleate *Polygonum*-type of embryo sac (Fig. 3D).

DISCUSSION

Considering it histologically, it was seen that the young placentae and the ovule primordial have a tunica-corpus organization. The tunica consists of 2 layers, namely the dermal layer or zone I and the subdermal layer or zone II. In the dermal layer, only anticlinal divisions occur, while in the subdermal anticlinal divisions are predominant. The corpus corresponds to the central area (zone III and zone IV), where no regular pattern is observed for cellular divisions. The tetrazonate feature of the ovule primordial is commonly seen in several angiosperm families [6,7](Bouman, 1984; Chehregani & Ranjbar et al,2008(. Periclinal division in zone III and IV of the placenta initiate the ovule in *O.sintenissii*. Other authors also have demonstrated the same process in several texa. [8,9 ,10 ,6 ,11](Bhandari et al. 1976, Bouman & Calis 1977, Bouman & Schier 1979, Bouman 1984, Venturelli & Bomtempi Jr. 1989). Despite the clear tetrazonate structure the ovule primordia of *O.sintenissii* has, the organization of the ovule primordia in *Jacaranda mimosifolia* is interpreted as bizonate, which is initiated by periclinal divisions in the 2nd cell layer of the placenta. Sometimes more than one archesporial cell can differentiate in the ovule primordia of *O.sintenissii*; however, only one of them can reach the MMC stage. Other fabaceae have also described the differentiation process of more than one archesporial cell in the same ovule[12,4 ,5](Guignard, 1881; Rembert, 1966, 1966, 1967). As Bouman [6](1984) pointed out, many texa present a multicellular archesporium, which is responsible for some cases of polyembryony or apomixes. The direct differentiation of the archesporial cell in a MMC observed in *Tabebuia pulcherrima* has also been reported in all the bignoniaceae investigated up to now. Integument ontogeny in bitegmic ovules is of great importance in the taxonomic study of the plant. Also a dermal origin of the integument was reported in *Datura stramonium* [13](Satina 1945) and *Scrophularia himalensis* [8](Bhandari et al. 1976). However, Bouman & Schier [10](1979) state that, in unitegmic/sympetalous plants, the integument does not always have an exclusively dermal origin, and can also be derived completely or partially from the subdermal layer. The ovule of *O.sintenissii* is bitegmic and crassnucellate. The dermal cells near the micropylar margin of the integument are usually considered homologous to the internal integument of the bitegmic ovule[9,10 ,6] (Bouman & Calis 1977, Bouman & Schier 1979, Bouman 1984). More usually seen in anatropous ovules, the funicular or basiovular part of the integument has a, much less visible development than the other side. In the latter, periclinal or oblique divisions of the inner cellular layers help increase the thickness of the integument, while the outer layer undergoes only anticlinial divisions. Bouman [14](1971) observed the same pattern of cellular divisions, in the solid external integument of the bitegmic ovule of Lilium.

In a previous study on flower biology of Papilioloideae, the ovule of *O.sintenissii* in the present study was reported to be basically anatropous, though it was not examined in depth. Having studied the longitudinal section, it can be indicated that the ovules are derived from ana-campylotropous forms[15] (Johri et al., 1992). The ovules show a single vascular bundle that crosses the funiculus and ends in the nucellus bas (chalaza) which is a common feature in most angiosperms, although the vascular penetration into one or both the integuments has been observed in some families such as legumes, [15,16 ,2] (Johri et al., 1992; Danilova, 2002; Lersten, 2004). The most common developmental pattern of the megagametophyte in Papilionoideae is the monosporic one [17,18 ,19 ,20 ,21] (Martin, 1914; Reeves, 1930; Johansson and Walles, 1993; Cameron and Prakash, 1994; Moco and Mariath, 2004), but in *Lupinus*, *Laburnum anagyroides*, *Pueraria lobata*, the bisporic pattern has also been reported [22](Rembert, 1969a) also in *Wisteria sinensis* [23](Rembert 1969b). The meiotic division of the megasporocyte always leads in a linear tetrad of megaspores, which only the chalazal megaspore grows to give rise to the megagametophyte. In all the fabaceae investigated up to now it was observed that the pattern may occasionally varition in tetrad, can be T-shaped or in an isobilateral arrangement, or a decussate arrangement. The results attained are in accordance with the studies of Guignard [12](1881) who reported that *Cydsus* megasporogenesis shows some variation with respect to the common pattern. Various researchers have reported this observation as the most typical model for some

other legumes among them *Vicia villosa* [23](Rembert, 1969b), while still other cases are observed in which this pattern is not typical, rather another alternative pattern is observed (megaspore tetrad formation), as in *Robinia pseudacacia* [22] (Rembert 1969a). However this model is not the only variation observed in leguminous plants, as for example, Rembert[4,5] (1966, 1967) indicated a bisporic model in *Wisteria sinensis* and Robinia pseudacacia, also Guignard [12](1881) in *Lupinus* polyphyllus. Moco and Mariath [21](2004) reported the embryo sac in *Lupinus* as a bisporic development, even though both the analysis of the results of Guignard [12](1881) and our results indicate a monosporic development for *O.sintenissii*. No sign of asymmetry was observed in the premeiotic MMC. However, there is a short delay of the second meiotic division in the dyad micropylar cell which can be interpreted as assign of polarity between the two cells, possibly related to the callose deposition in the transverse wall between them. In *O.sintenissii* the second division in the chalazal cell, and not rarely also in the micropylar cell, is sharply unequal. At the tetrad stage, the largest volume of the distal micropylar megaspore relatively to the two intermediate ones is probably associated to its largest contact surface with the nucellar epidermis. The embryo sac formation in this species follows the *Polygonum* model without any variation from megaspore to eight-nucleate phase, and final (eight-nucleate) phases show half of the nuclei towards the chalazal end and the other half towards the micropylar end, separated by a large central vacuole that, according to Folsom and Cass [24](1998, 1990) and Bittencourt and Mariath [25] (2002), limits nuclear movements and thus controls ontogenetic events. In conclusion, ovule primordia in *O. sintenissii* have a tetra-zonate structure, in which two integuments are originated from the dermal and subdermal layers. These features may be important at generic level of fabaceae taxonomy because a different condition –i.e., bizonate with a dermal origin of the integument- was reported in Jacaranda[26] (Galati & Strittmatter 1999).

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