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Full Length Article

Developmental and Cytochemical Features of Female Gametophyte in Endemic *Lathyrus undulatus* (Fabaceae)

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Abstract

The presented study describes developmental and cytochemical features of female gametophyte in *Lathyrus undulatus* Boiss. (Fabaceae), which belongs to subfamily Papilionoideae and is endemic to northwestern Turkey. The ovary is monocarpellate and the mature ovule is anatropous, bitegmic and crassinucellate. Differentiation of the outer integument determines a Y-shaped micropyle aperture. The nucellar epidermis is poorly developed. The inner parietal layers of the integument differentiate into a thin layer of endothelium. The hypodermally oriented archesporium is distinguished as one cell. Archesporium produces a parietal cell and a primary sporogenous cell. The primary sporogenous cell enlarges to form megasporocyte. After meiotic division, megaspores organize in an isobilateral manner of tetrad. Only one megaspore functions and it is one of the chalazal members of the tetrad. The functional megaspore develops into an embryo sac, which gives rise to a Polygonum type. The synergids lying above the egg cell are typical in structure. The antipodals are ephemeral and degenerate earlier without leaving any remnants. Embryo development is of Onagrad type. Suspensor degenerates at the globular embryo stage. The primary endosperm nucleus divides mitotically in a free nuclear manner and it is rich for insoluble polysaccharides and protein. © 2013 Friends Science Publishers

Keywords: *Lathyrus undulatus;* Megasporogenesis; Megagametogenesis; Embryo; Endosperma

Introduction

The genus *Lathyrus* L. (Fabaceae) comprises approximately 200 species, most of which are annual and perennial plants, predominantly centered in the Mediterranean region. *Lathyrus* is represented by 78 *Lathyrus* taxa, 24 of which are endemic to Turkey (Davis, 1970; 1988; Güneş and Çırpıcı, 2008; 2011). *Lathyrus* species have high ecological and economical importance including drought resistance, erosion preventation, food, agricultural processes and ornamental purposes (Güneş, 2011).

Anatomical, palynological, caryological and seed morphological studies on some species of *Lathyrus* have been performed to elucidate the phylogenetic relationship of *Lathyrus* genus within the subfamily Papilionoideae (Perveen and Qaiser, 1998; Tosheva *et al.*, 2004; Tosheva and Tonkov, 2005; Abou-El-Enain *et al.*, 2007; Güneş and Çırpıcı, 2008; 2010; Güneş and Aytuğ, 2010).

It has been known that there are some taxonomic problems between the members of this genus that can not be solved by anatomical and morphological characters (Mantar *et al*., 2003). However, despite the usefulness of the embryological characters in this type of analysis, there are only a few studies on male and female gametophyte development in *Lathyrus* genus (Latter, 1925; Rembert, 1969; Davies and Williams, 1985). Recently, developmental, cytochemistry and programmed cell death studies were reported on the anthers of *Lathyrus undulatus* in detail (Vardar and Ünal, 2011a; 2011b; 2011c; 2012).

Besides, the developmental features of megagametophyte and embryo seem to be very significant not only for systematic comparisons, but also for the knowledge of development and fertilization process. To the best of our knowledge, only the megasporogenesis of *L. latifolius* (Rembert, 1969a) and *L. sativus* (Roy, 1933) were carried out and there is no previous data available on the megagametophyte and embryo development of *L. undulatus* as well as the other members of *Lathyrus* genus.

The present study reports the first observations on the development and cytochemistry of ovule in *L. undulatus* Boiss. (Fabaceae), which belongs to Papilionoideae subfamily and is endemic to northwestern Turkey. This study will provide a detailed understanding of the events that leads to embryo formation. Information on the development of the female reproductive structures in *L. undulatus* will advance our understanding of its reproductive process, and contribute to understand taxonomic relationship with closely related taxa within the Papilionoideae.

Materials and Methods

Flower buds of *Lathyrus undulatus* Boiss. (Fabaceae) growing in natural habitats in the vicinity of Beykoz-

İstanbul (Turkey) was collected in March-April 2008. Flower buds were fixed in acetic:alcohol (1:3, v/v) for 24 h at room temperature. After dehydration in a graded series of ethanol, the material was embedded in paraffin. Sections (8-–10 µm) were cut using a Leica RM2125RT microtome and stained with Delafield's hematoxylin.

Insoluble polysaccharides were localized according to periodic acid-Schiff's (PAS) method of Feder and O'Brien (1968). The sections were immersed in a solution of 1% periodic acid dissolved in 96% ethanol for 20 min. Staining in Schiff's reagent was carried out for 30 min in the dark. The presence of carbohydrate in the tissue is indicated by the appearance of a purplish-red color. Proteins were localized according to a method of Fisher (1968). The sections were stained with 0.2% Coomassie Brilliant Blue dissolved in a methanolic solution, methanol:acetic acid: water (5:1:4), for 30 min at 60°C. The presence of protein is indicated by the appearance of a blue color.

The sections photographed with the ProgRes Capture Pro 2.6 software, assisted by a Jenoptik 122CU color camera and an Olympus BX-51 microscope.

Results

In *L. undulatus* the ovary is monocarpellate. The mature ovule is anatropous, bitegmic and crassinucellate. The inner integument initiates first, and then the outer integument develops into a small protuberance at the megaspore mother cell stage. Throughout the development the outer integument becomes multilayered and swells at the micropylar region where it consists of 9–10 layers of cells. The differentiation of the outer integument, in the proximal flank of the primordium determines a Y-shaped micropyle aperture (Fig. 1a). Besides, the inner integument remains uniformly two layered which include numerous starch grains at the stage of mature embryo sac. The asymmetrical integument growth creates the anatropous curvature. The nucellar epidermis is poorly developed and composed of 4–5 layers of cuboidal cells. The inner parietal layers of the integument differentiate into a thin layer of endothelium with cuboidal cells comprises strong PAS positive reaction (Fig. 1b, c).

Megasporogenesis is initiated by the development of an archesporium hypodermally oriented in the nucellus. The archesporium is distinguished as one cell, which represents a large volume, dense cytoplasm and distinct nucleolus. Development continues with the mitotic division of the archesporium cell, producing a parietal cell and a primary sporogenous cell (Fig. 2a). Parietal cell remains undivided in the nucellus. The primary sporogenous cell enlarges to form the megasporocyte (megaspore mother cell-MMC) (Fig. 2b). The MMC undergoes meiosis I to form equally-sized dyad. In *L. undulatus* as distinct from the common at the end of meiosis I the wall formation is longitidunal (Fig. 2c-f). The meiotic division in the MMC is synchronic with the microspore mother cells.

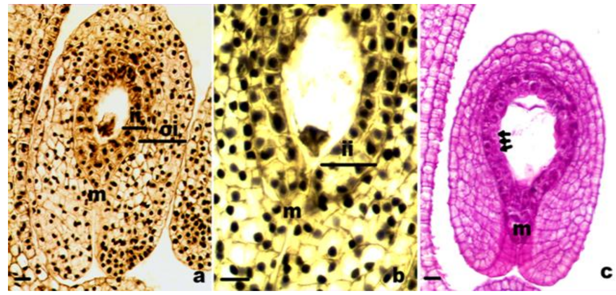

Fig. 1: Ovular structures of *Lathyrus undulatus*. (a) Positions of micropyle, inner and outer integument stained with hematoxylin. (b) Two layered inner integument and Y-shaped micropyle aperture. (c) Inner integument differentiated into endothelium (arrows). Note the strong PAS positive reaction and starch grains. ii: Inner integument, m: Micropyle, oi: Outer integument. Bars =10 µm

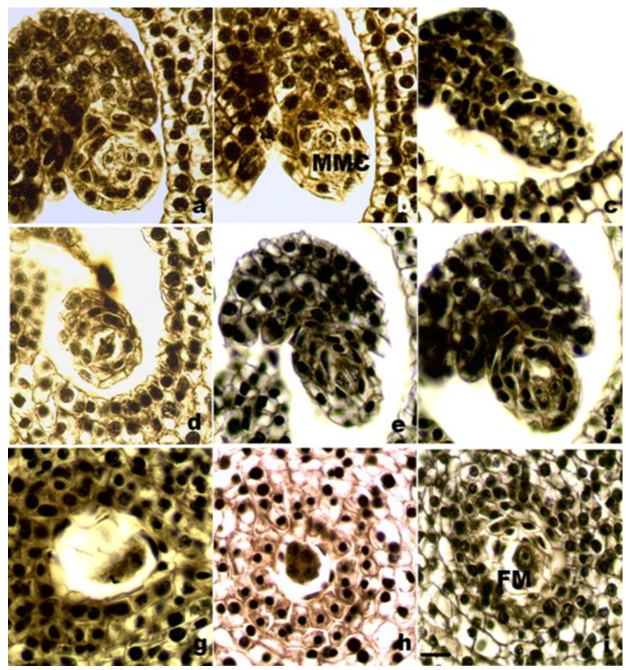

Fig. 2: Megasporogenesis in *Lathyrus undulatus*. (a) A parietal cell and a primary sporogenous cell. (b) Megaspore mother cell (MMC). (c) Prophase I. (d) Metaphase I. (e) Telophase I. (f) Prophase II. (g) Metaphase II. (h) Isobilateral tetrad. (i) Functional megaspore (FM) at chalaza. Bar in (i) is 10 µm and also applies to in (a)-(h)

At the conclusion of the meiotic divisions in the ovule of *L. undulatus*, an unusual arrangement of tetrad is formed (Fig. 2g, h). The megaspores organize in an isobilateral manner of tetrad. Only one megaspore functions and it is always one of the two larger chalazal members of the tetrad (Fig. 2i). At about the functional megaspore stage the ovule is completely anatropous.

The functional megaspore develops into an embryo sac (ES), which increases in length, extending into the micropylar region. Mitotic divisions in the functional megaspore result in 2, 4 and 8-nucleate ES (Fig. 3a-c). These nuclei reorganize to give rise to a Polygonum type of ES. Three nuclei from the micropylar quartet develop into an egg apparatus consisting of an egg and two synergids, which are PAS positive (Fig. 3d, e). The synergids lying above the egg cell are typical in structure. The antipodals, formed from the three nucleus of chalazal quartet, are situated on the periphery at the chalazal end of the ES (Fig. 3f). The antipodals are ephemeral and degenerate earlier without leaving any remnants. The fourth nuclei from the micropylar and chalazal quartet form the polar nuclei (Fig. 3g). The two polar nuclei fuse to form a secondary nucleus before fertilization, which is situated by the side of the egg. The central cell contains PAS positive granules (Fig. 3h). After fertilization, the synergids degenerate simultaneously (Fig. 3i), and secondary nucleus gives rise to the primary endosperm nucleus.

Embryo development is of Onagrad type. Zygote presents at the micropylar pole of the ES and smaller than the egg cell (Fig. 4a). The volume decrement is due to the reduction of vacuole. The first division of zygote is transverse giving rise to an apical cell *ca* and a basal cell *cb* almost equal in size (Fig. 4b). Apical cell *ca* divides transversely. The two daughter cells of *ca* commonly divide by a vertical wall and constitues to form a proembryo of four cells. Subsequent divisions give rise to globular embryo. Basal cell *cb* divides also transversely forming two superimposed cells (Fig. 4c, d). The basal cell constitutes the suspensor, which becomes larger. The suspensor persists until globular embryo and then degenerates (Fig. 4e, f).

The primary endosperm nucleus prior to division in the zygote divides mitotically in a free nuclear manner. Free nuclei remain embedded in a cytoplasmic sheet around the central vacuole (Fig. 5a). The endosperm is rich for insoluble polysaccharides and protein carried out by PAS and Coomassie Brilliant Blue, respectively (Fig. 5b, c).

Discussion

The reproductive and embryological knowledge of economically important plants, belonging to Fabaceae, provides useful data in relation to the fields of cell biology, reproductive ecology and taxonomy and for purposes related to seed production and cross-breeding. According to embryological data (Johri *et al.*, 1992), considerable information is available on this family, with greater emphasis on subfamily Papilionoideae. Although anatomy, pollen morphology and the chromosome number were most widely analyzed in *Lathyrus* genus, which belongs to Papilionoideae, male and female gametophyte development was not noticeable. Recently development, cytochemistry and programmed cell death studies have been performed on the anthers of *L. undulatus* in detail (Vardar and Ünal,

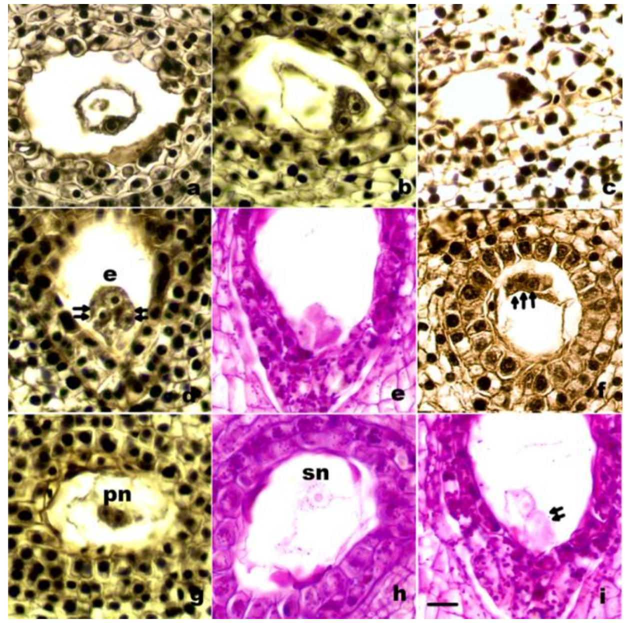

Fig. 3: Development of embryo sac (ES) in *Lathyrus undulatus*. (a) 2-nucleate ES (one of the nuclei is observed). (b) 4-nucleate ES (two of the nuclei is observed). (c) Unorganized 8-nucleate ES (four of the nuclei is observed). (d) Egg apparatus with an egg (e) and two synergids (double arrows). (e) PAS positive egg apparatus. (f) Antipodals (arrow). (g) Two polar nucleus (pn). (h) Secondary nucleus (sn) and PAS positive granules in central cell. (i) Remained synergid (double arrows) after fertilization. Bar in (i) is 10 µm and also applies to in (a)-(h)

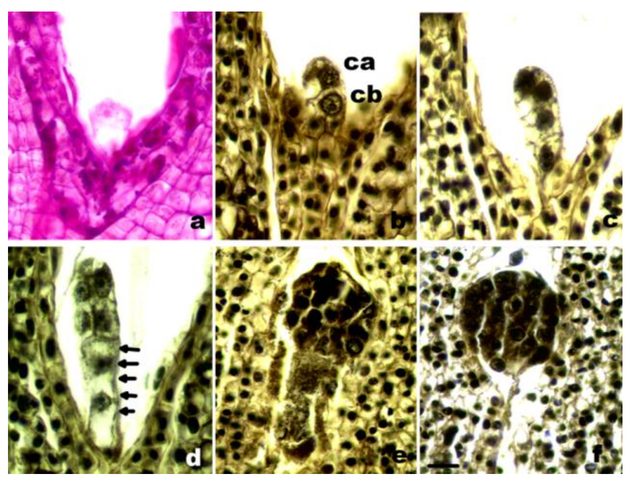

Fig. 4: Embryo development of *Lathyrus undulatus.* (a) Zygote. (b) Two-celled proembryo; apical cell (ca), basal cell (cb). (c) Four-celled linear proembryo. (d) Basal cell divides and constitutes to suspensor (arrows) (e) Globular embryo with large suspensor. (f) Globular embryo with degenerated suspensor. Bar in (f) is 10 µm and also applies to in (a)-(e)

2011a; 2011b; 2011c; 2012). Besides, there are only a few studies on female gametophyte development, which consist only of megasporogenesis in *Lathyrus* genus (Latter, 1925; Rembert, 1969a; Davies and Williams, 1985).

The ovule characters are important particularly for the Fabaceae family such as ovule shape and type, the integuments and the participation of integuments in micropyle formation. The ovule of *L. undulatus* is anatropous, bitegmic and crassinucellate consistent with Fabaceae family. Davis (1966) indicated that the micropyle is formed by both integuments and often markedly zig-zag in Papilionaceae (synonym of Fabaceae). Although micropyle is generated by both of the integuments similar with the other family members, it is distinctively Y-shaped in *L. undulatus*, which was also reported in *Adesmia latifolia* (Moço and Mariath, 2003). Besides the inner integument consists of two cell layers in *L. undulatus*, while the outer integument comprises of several layers as common in Fabaceae (Hindmarsh, 1964; Deshpande and Bhasin, 1974; Rembert, 1977; Ashrafunnisa and Pullaiah, 1994; 1999; Moço and Mariath, 2003). As it was observed in the presented study, the nucellus of the Fabaceae ovules is crassinucellate (Prakash, 1987), and which has been considered to be the primitive condition in angiosperms (Sporne, 1969).

Rembert (1969a) compared megasporogenesis of 16 species of Papilionaceae family and indicated that in this family an archesporium, whether multicellular or a single cell, is characteristically hypodermal. The researcher explained that in hypodermal development a parietal cell and a primary sporogenous cell are produced in 14 of the 16 species and the primary sporogenous cell enlarges to form a single megasporocyte in these 14 species, as it was presented in *L. undulatus*.

Rembert (1966; 1969a; 1969b; 1971) used the marked variability in the megaspore tetrads to make phylogenetic hypotheses in Fabaceae. The author described the occurence of ten types of tetrads (linear with chalazal or epichalazal functional megaspore, T-shaped, oblique linear, inverted T-shape, anisobilateral, triad and three different types of bisporic patterns) and supposed evolutionary lines with in the family. Roy (1933) reported T-shaped tetrad for *L. sativus*. Besides, a related species *L. latifolius* displays a unique pattern of megaspore tetrads as anisobilateral (Rembert, 1969a). According to our results, as distinct from the other *Lathyrus* species *L. undulatus* exhibited an isobilateral tetrad.

In all other species of Papilionaceae it is the chalazal megaspore that functions in megagametogenesis and in no case is more than one megaspore known to function in this family. In general chalazal megaspore of tetrad develops into Polygonum type embryo sac (Davis, 1966; Rembert, 1969a). These are also the cases in *L. undulatus*.

The monosporic, Polygonum type of female gametophyte is typically a seven-celled structure at maturity. However, this structure may be reduced by cell death.

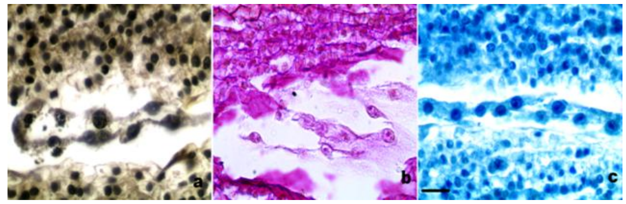

Fig. 5: Free nuclear endosperm of *Lathyrus undulatus* stained with hematoxylin (a), PAS (b) and Coomassie brilliant blue (c). Bar in (c) is 10 µm and also applies to in (a)-(b)

In *Arabidopsis* the antipodal cells underwent cell death before fertilization (Yadegari and Drews, 2004). Similarly ephemeral antipodals were observed in *L. undulatus* which is consistent with the Papilionaceae (Davis, 1966). Davis (1966) represented that embryogeny conforms either to the Onagrad, Asterad or Caryophyllad type and the suspensor shows great variation between the species in Papilionaceae. *L. undulatus* shows Onagrad type embryogeny and large suspansor, which persists until globular embryo.

It has been reported that endosperm formation is nuclear in Papilionaceae (Davis, 1966). In *L. annuus* and *Stylosanthes mucronata* wall formation is suppressed and the endosperm remains free-nuclear (Davis, 1966). Similar was the case for *L. undulatus*.

In conclusion; our data provide a new look at the aspect of sexual reproductive potential of *L. undulatus* in the genus *Lathyrus*. On the other hand, developmental and cytochemical features will be the object of fertilization and embryo growth investigations in *Lathyrus* thereby in subfamily Papilionoideae. Moreover, embryo sac features will provide useful characters in assessing relationships within this genus and family.

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