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# ANATOMY AND ONTOGENY OF *ASTRAGALUS REMOTIJUGUS* BOISS. & HOHEN. SEED

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**Abstract**: The aim of this study was to describe the anatomy and ontogeny of *Astragalus remotijugus* Boiss. & Hohen. seed using the usual techniques. The ovules are campilotropous, crassinucelate, and bitegmic. The nucellus cells disappear during an early stage of development. The following processes occur during integument development: anticlinal divisions and formation of palisade cells in the exotesta; predominantly periclinal divisions and cell expansion in the mesotesta; and endotesta differentiates in to an endothelium layer with thick and cubic in shape cells. The primary endosperm nucleus divides before the zygote nucleus, forming a nuclear endosperm. Endosperm cellularization begins when the embryo has developed the late globular stage. The embryological characters of *A. remotijugus* Boiss. & Hohen. are compared with other species of Fabaceae and those of other species of *Astragalus*. The remarked characteristic of the embryo was presence large suspensor with six columns of cells, and its large haustorial cells. Another difference in the development of *A. remotijugus* Boiss. & Hohen. seed was presence additional embryo at the globular stage that probably was developed from suspensor cells.

**Keywords**: embryogenesis, Fabaceae, polyembryony, seed coat, suspensor

## Introduction

The Fabaceae family consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families [POHILL & al. 1981; JUDD & al. 1999]. *Astragalus* L., with about 3000 species worldwide, is the largest genus of flowering plants. The high variation of morphological characters has made infrageneric classification uncertain and problematic [SANDERSON & LISTON, 1995; SANDERSON & WOJCIECHOWSKI, 1996; WOJCIECHOWSKI & al. 1999; ZARRE, 2000; KAZEMPOUR-OSALOO & al. 2003, 2005]. It is important to emphasize that seed morphology usually shows little phenotypic plasticity. On the other hand, embryological characters, usually constant in the genera, function as a significant indicator of taxonomic affinity [RIAHI & al. 2003; RIAHI & ZARRE, 2009; FARHANGISABET & al. 2011]. According to these authors, there are few descriptive and ontogenetic studies on seed structure, which makes speculating about evaluative trends affecting seeds very difficult. Therefore, in the present study we investigate detailed embryology in *Astragalus remotijugus* Boiss. & Hohen. of *Astragalus* subgenera and *Caprini* section. The reason for selection this species is distribution at near of study place, because flower bud and fruits

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were collected at different stage. Also *A. remotijugus* Boiss. & Hohen. has big seed with relatively thin coat that is appropriate for section. The aim of this study was to describe the anatomy and ontogeny of *Astragalus remotijugus* Boiss. & Hohen. seed, emphasizing its testa, suspensor and endosperm structure.

## Materials and methods

Flower buds, flowers and fruits were collected at different stage in May 2009 and 2010 from Alborze mountains north of Tehran province (Jajerood and Roodehen), Iran. Then fixed in formalin-glacial acetic acid and 70% ethanol ($FAA_{70}$, 5:5:90), stored in 70% ethanol, embedded in paraffin and sectioned at 6-8 µm with LEICA RM2255 rotary microtome. Staining was carried out using MICROM HMS70 and the periodic acid Schiff (PAS) and Meyer's Hematoxylin techniques. Sections were viewed with an OLYMPUS CX-31 light microscope.

## Results

*Astragalus remotijugus* Boiss. & Hohen. ovules are campilotropous, crassinucelate, and bitegmic (Fig. 1A). The outer integument shows three to five layers of isodiametric cells; the inner integument has two to three layers. The micropyle is zigzag, with a larger number of cell layers in the exostome. The embryo sac cone- shaped just prior to fertilization in sagittal section. The embryo sac is 8- nucleate (7- celled) and of the Polygonum- type, which composed of 3 antipodal cells, 2 polar nuclei and a 3- celled egg apparatus. The egg apparatus consists of the egg cell and synergids (Fig. 1B). At the chalazal end of the embryo sac the nucellus consists of one or two cell layers as nucellus cap (Fig. 1C). The innermost of inner integument cell layer, the so called endothelium, is characterized by a thick layer of cuticle at the side adjacent to the embryo sac and large nucleated cells. These cells are cubic in shape (Fig. 1D).

Many alterations occur in the beginning of seed ontogeny, when the embryo is in proembryo stage (Fig. 1E). The first endosperm nucleus possesses a single large nucleolus. Few divisions of the primary endosperm nucleus occur before the first division of the zygote. Endosperm formation is free nuclear, that is, not followed by cytokinesis. In this manner the endosperm forms first at the micropylar chamber, then spreads towards the chalazal chamber as peripheral layer and remains free- nuclear for a while (Fig. 1E). The embryo development is the Onagrad type. Embryo differentiates in to a globular embryo proper and suspensor (Fig. 1F). Development of cellular endosperm begins during the late globular stage and showing great cytoplasmic density in two or three external peripheral layers (Fig. 2A). During the globular stage, the endosperm haustorium appears at the chalazal end. It is narrow and shows a tubular structure (Fig. 2A). The cells of the outer integument layer elongate and begin to differentiate to form the palisade layer (macrosclereid cells) characteristic for the testa of Fabaceae. The differentiation of the cells in this layer starts close to the hilum. The exotesta (palisade layer) is composed of a single cell layer, except for the region surrounding the hilum which is two layers thick (Fig. 2A). This layer is denser than the other layers, with cubic cells showing slight radial elongation. The mesotesta has two hypodermal layers which are composed of thin walled cells. Beneath this is endotesta with 3-4 parenchymatous cell layers with thick walls (Fig. 2A). The suspensor is large and is composed of six cell columns with several (Fig. 2B, E) inflated cells embedded in maternal tissue (Fig. 2B). After formation of cotyledon primordia the embryo takes its heart- shaped form. By enlargement of cotyledons and embryo axis, the torpedo- shaped embryo grows in to the cellularized endosperm (Fig. 2C). In 10 % of ovule sections in globular embryo stage is an additional embryos that probably is originated from the suspensor cells (Fig. 2D, E). However, in 5% of ovules of *A. remotijugus* Boiss. & Hohen. there are more zygote to recognize, which represents most probably on abnormality. In such anomalous ovules three are 3 zygote, composed of polar nuclei, main egg and zygote cell that seem to be derived from one of the synergid (Fig. 2F).

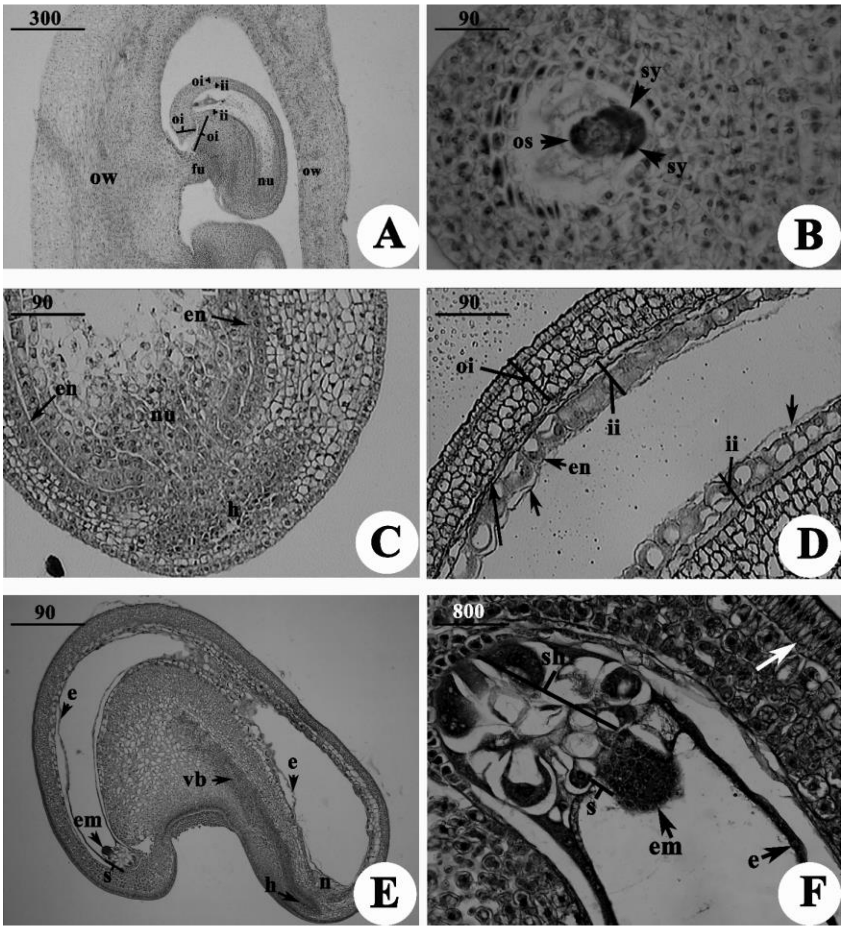


**Fig. 1.** A. Fertilized ovule in longitudinal section; the embryo sac (es) begins to be horse- shoe shaped; fu (funicle); ii (inner integument); oi (outer integument); nu (nucellus); ow (ovary). B. Transverse section of ovule before fertilization showing egg apparatus; os (egg); sy (synergid). C. Chalazal end of the embryo sac and nucellus cap; en (endothelium); h (hypostase); nu (nucellus). D. Detail of integument and endothelium, arrows indicate the cuticle layers. E. Section of the ovule showing the proembryo; coenocytic endosperm (e) forms a thin sheath around the embryo sac; e (endosperm); em (embryo); h (hypostase); nu (nucellus); s (suspensor); vb (vascular bundle). F. Detail of embryo with suspensor (s) and suspensor haustorium (sh); white arrow indicates the palisade layer; e (endosperm); em (embryo). Bars in µm.

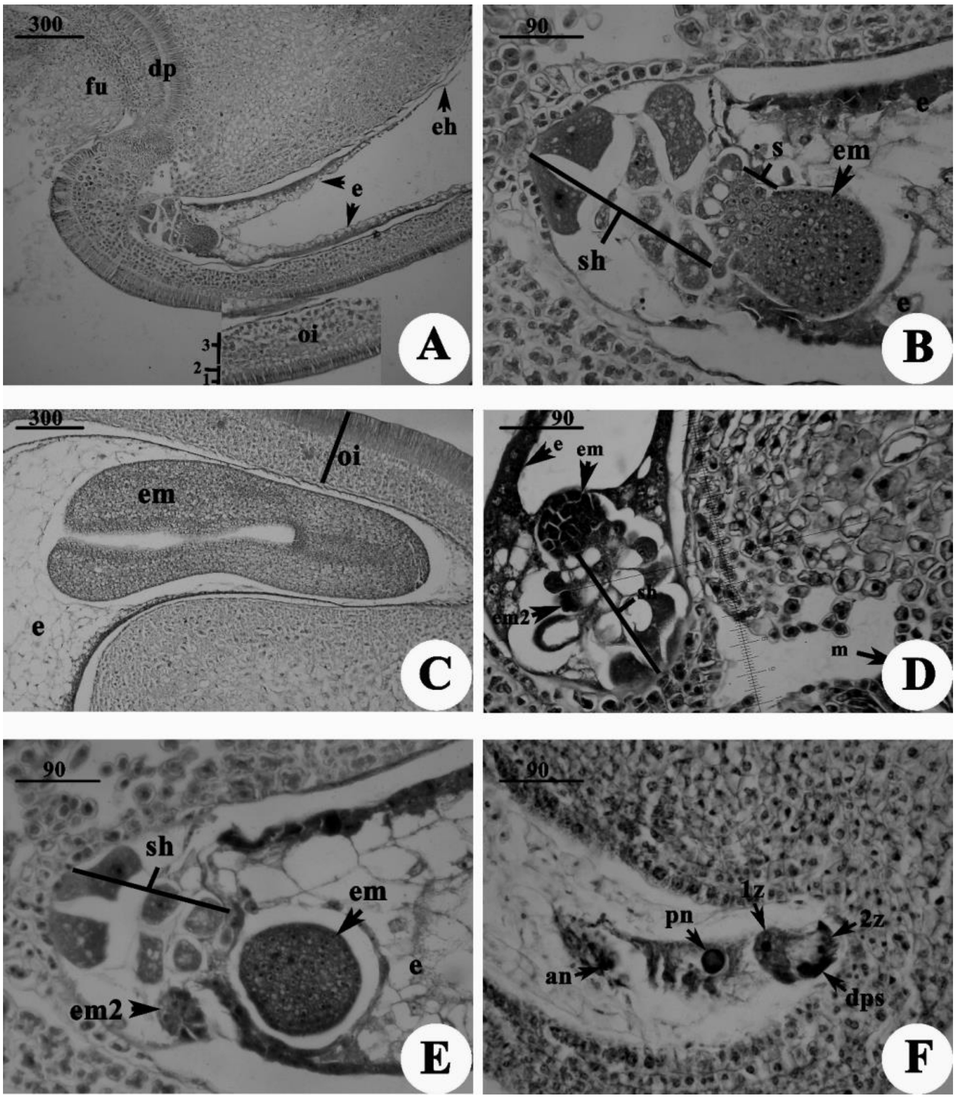


**Fig. 2.** A. General view of seed with detail the different parts of outer integument; e (endosperm); eh (haustorial endosperm); fu (funicle); dp (double palisade layer); oi (outer integument); vb (vascular bundle). B. Detail the embryo, suspensor (s) and suspensor haustorium (sh); glass arrow show peripheral endospermal cells are rich in cytoplasmic content; e (endosperm); em (embryo); suspensor (s); suspensor haustorium (sh). C. Cotyledon stage of the embryo (em); endospermal cells (e) are thin walled and begin to degerate; oi (outer integument). D. Early globular embryo (em) with additional embryo (em2) and coenocytic endosperm (e); suspensor haustorium (sh). E. Late globular embryo (em) with additional embryo (em2); e (endosperm); suspensor haustorium (sh). F. An abnormal young embryo sac including normal zygote (1z); fertilized synergid (2z); degenerative persistent synergid (dps); fertilized polar nuclei (pn) and antipodal cells (an). Bars in µm.

## Discussion

**Comparison with Fabaceae:** Fabaceae seeds are typically testal, produced by bitegmic ovules, in which inner integument reduction frequently occurs [EAMES & MAC DANIEL, 1953; CORNER, 1951, 1976; LERSTEN, 1983; PRAKASH, 1987; AKHALKATSI & al. 1988; GVALADE & AKHALKATSI, 1996; OLIVEIRA & PAIVA, 2005]. In the Fabaceae endosperm varies from abundant to absent [CORNER, 1951, 1976; GUNN, 1981]. The studies by De CANDOLLE (1825) following which Fabaceae embryo structure have been considered of major important, resulted in the division of the Fabaceae in to two great subfamilies based on embryo axis curvature (Curvembriae and Rectembriae). The first subfamily includes the Faboideae and the second, the Caesalpinioideae and Mimosoideae. Although embryo axis curvature is presently not regarded as the best character for primary divisions in the family, it indicates better protection for the radical and may be one of a set of seed characters (especially hilar characters) used to separate the Faboideae from the other subfamilies [GUNN, 1981]. EAMES & MAC DANIELS (1953) generalized about the occurrence of complete absorption of the inner integument and nucellus during Fabaceae seed development. CORNER (1976) reported that because the tegument is crushed at maturity, it does not contribute to the seed- coat. Some species of the three Fabaceae subfamilies, in which the tegmen is absent in the mature structure, have been illustrated by CORNER (1951, 1976). BOESEWINKEL & BOUMAN (1984) also reported that the inner integument of Fabaceae is either crushed or reabsorbed. Several cases in the literature confirm this observation, e. g. *Indigofera enneaphylla* [DESHPANDE & UNTAWALE, 1971], *Indigofera parviflora* [MANNING & VAN STADEN, 1987], *Capaifera langsdorffi* [CRESTANA & BELTRATI, 1988], *Inga fagifolia* [OLIVEIRA & BELTRATI, 1993] and *Tipuana tipu* [MARTINS & OLIVEIRA, 2001]. Thus inner integument reduction is usual in Fabaceae, as is *Astragalus remotijugus* Boiss. & Hohen. seed, since has been demonstrated. Another distinguishing characteristic is embryo axis curvature in *A. remotijugus* Boiss. & Hohen.

**Comparison with other *Astragalus* species:** The embryo sac is Cone- shaped but finally differentiates in to the typical horse- shoe- shaped and this character is the same to other *Astragalus* species [AKHALKATSI & al. 1988; GVALADE & AKHALKATSI, 1996; RIAHI & al. 2003; RIAHI & ZARRE, 2009; FARHANGISABET & al. 2011]. The nucellus begins to degenerate when the embryo sac is formed. At the early globular embryo stage the nucellus is completely degenerated, although in some other *Astragalus* [RIAHI & al. 2003; RIAHI & ZARRE, 2005; FARHANGISABET & al. 2011] the disintegration of nucellus is completed at the formation of the proembryo.

The integuments development in *A. remotijugus* Boiss. & Hohen. was the same shown in other species of *Astragalus* [RIAHI & al. 2003; RIAHI & ZARRE, 2005; FARHANGISABET & al. 2011]. Differentiation of the integuments first occurs in the inner integument with the formation of the endothelium at an early stage of development. Then the inner integument degenerates at heart stage. The outer integument begins to take on the characteristic morphology of mature testa at proembryo stage, but reaches its maximal width and differentiation at the heart- shaped embryo stage. The main patterns in the development of endosperm in *A. remotijugus* Boiss. & Hohen. is similar with other cases recorded in *Astragalus*, i. e. *A. caucasicus* [AKHALKATSI & al. 1988], *A. denudatus* and *A. microcephalus* [GVALADZE & AKHALKATSI, 1996], *A. demavendicus* and *A.*

*latifolius* [RIAHI & al. 2003], *A. cemerinus* and *A. ruscifolius* [RIAHI & ZARRE, 2009], *A. eriocarpus*, *A. glaucacanthus*, *A. chrysostachys* and *A. compactus* [FARHANGISABET & al. 2011].

The shape of the embryo at different stages in *A. remotijugus* Boiss. & Hohen. is similar to other studied species of *Astragalus* [AKHALKATSI & al. 1988; GVALADZE & AKHALKATSI, 1996; RIAHI & al. 2003; RIAHI & ZARRE, 2009; FARHANGISABET & al. 2011], but considerable differences exist in the embryogenesis of this species that can be studied.

A typical characteristic of embryo is large suspensor which is composed of six columns of cells with several (10 to 15) inflated cells as haustorial cells. In comparing with other studies about *Astragalus* species, this type of suspensor is the largest type in studied *Astragalus* specieses [AKHALKATSI & al. 1988; GVALADZE & AKHALKATSI, 1996; RIAHI & al. 2003; RIAHI & ZARRE, 2009; FARHANGISABET & al. 2011].

An important character in this species is presence additional embryo at the globular stage. In this manner seems that additional embryo is developing from suspensor cells. The suspensor cells appear to be polyploidy, and the localization of the additional embryo indicates that the embryo arose from suspensor cells. According to articles published, this phenomenon is called polyembryony [BOTYGINA & VINOGRADOVA, 2007; CZAPIK, 1999]. In *Astragalus* genus this type of formation additional embryos has been described for the first time as well as some type of polyembryony. Another kind of polyembryony as perimitotic type of fertilization was reported in *A. caucasicud* [AKHALKATSI & al. 1988].

## Conclusions

As a result of our work it becomes clear that such characteristics in the development of the ovule are most probably appropriate for separating taxa at species rank. More such studies on other species of *Astragalus*, can reveal the characteristics and differences useful for separating higher taxonomic ranks.

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