# MEGAGAMETOPHYTE AND EMBRYO DEVELOPMENT IN FIVE SPECIES OF ASTRAGALUS (FABACEAE)

## M. Farhangisabet, A. Majd, T. Nejadsattari, A.A. Maassomi & A. Mazouji

The development of the megagametophyte, embryo and the endosperm in *Astragalus eriocarpus* (sect. *Malacothrix*), *A. glaucacanthus* (sect. *Poterion), A. remotijugus* (sect. *Caprini*), *A. chrysostachys* (sect. *Hymenostegis*) and *A. compactus* (sect. *Rhacophorus*) were examined. The ovule are campylotropous, bitegmic and crassinucellate; integuments form a zig-zag shaped microphyle. The embryogenesis was similar in the species. Megagametophyte development follows the monosporic *Polygonum* type and the embryo development follows the Onagrad type. The fusion of the polar nuclei, development of the endosperm, digestion of the nucellus, seed abortion and suspensor development show major differences among studied species. Furthermore, the size and shape of the suspensor is quite variable among the five species. These characters are compared with the other species of *Astragalus* and also their phylogenetic significance is discussed. Also we describe a case of polyembryony for the first time in *A. remotijugus*.

*Mojgan Farhangisabet (correspondence, mfarhangis@yahoo.com) and Taher Nejadsattari, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, P. O. Box: 14515/775.- Ahmad Majd, Islamic Azad University, North Tehran Branch, Faculty of Science, Department of Biology, Tehran, Iran.- Ali Asghar Maassomi, Research Institute of Forests and Rangelands, Department of Botany, Tehran Iran.- Ali Mazouji, Islamic Azad University, Roodehen Branch, Department of Biology, Roodehen, Iran.*

تکوین رویان و کیسه رویانی در پنج گونه از گونهای ایران

**مژگان فرهنگی ثابت**، دانش آموخته دانشگاه آزاد اسلامی واحد علوم و تحقیقات تهران.

**احمد مجد**، استاد دانشگاه آزاد اسلامی واحد تهران شمال.

**طاهر نژاد ستاری**، دانشیار دانشگاه آزاد اسلامی واحد علوم و تحقیقات تهران.

**علی اصغر معصومی**، استاد پژوهش موسسه تحقیقات جنگلها و مراتع کشور.

**علی مازوجی**، استادیار دانشگاه آزاد اسلامی واحد رودهن.

تکوین رویان، کیسه رویانی و آندوسپرم در (Astragalus eriocarpus (sect. Malacothrix) A. glaucacanthus (sect. Poterion) A. remotijugus (sect. Caprini) A. chrysostachys (sect. Hymenostegis) و A. compactus (sect. Rhacophorus) بررسی شده است. تخمکها در هر پنج گونه نیمه واژگون هستند و بافت خورش توسط دو پوسته احاطه میشود. سفت به واسطه رشد نامتقارن پوسته خارجی، زیگزاکی است. الگوی کلی رویانزایی در هر پنج گونه مشابه یکدیگر است. در هر پنج گونه تکوین کیسه رویانی از نوع علف هفت بند و تکوین رویان از نوع گل مغربی است. اختلافات اصلی در رویانزایی این گونهها، زمان تلفیق هستههای قطبی، نمو آندوسپرم، هضم خورش، سقط رویان و از همه مهمتر نمو آویز میباشد. اندازه و شکل آویز در هر پنج گونه متفاوت است، این نتیجه با نتایج حاصل از مطالعات مولکولی در گون مقایسه شده است. در پژوهش حاضر، در گونه A. remotijugus برای اولین بار چندرویانی گزارش شده است.

# INTRODUCTION

From the Linnaes time until now, the genus *Astragalus* has been the subject of a good deal of nomenclatural changes. *Astagalus* L. (*Fabaceae*, *Papilionoideae*) is the largest genus of the flowering plants with about 2000-3000 species (Maassoumi, 1998; Podlech, 1982), and a major center of diversity is Iran with about 800 species. Subgeneric classification of the genus has been a matter of controversy. Podlech (1982) reduced the eight subgenera proposed initially in the genus (Bunge, 1868) to two *Astragalus* and *Cercidothrix*. Several molecular systematic studies, such as RFLP (Restriction Fragment Length Polymorphism) analysis (Sanderson & Doyle, 1993) and analysis of different chloroplast (e.g., $rpoC_1$ and $rpoC_2$ : Liston, 1992 or ndhF: Kazempour Osaloo et al., 2003) and nuclear genes (e.g., Nuclear Ribosomal DNA ITS: Wojciechowski et al., 1999; Kazempour Osaloo et al., 2003 and 2005), have explained the phylogenetic relationships within *Astragalus*, but only a few groups found support from previous systematic studies. There are also some efforts on reconstructing phylogeny of different groups of *Astragalus* based on morphological characters, indicating high level of homoplasy for these characters (Sanderson, 1987; Zarre, 2000).

Embryological studies in *Papilionoideae* (Rembert, 1969; Plaser, 1975; Laresten, 1983; Prakash, 1987; Cameron & Prakash, 1994; Ashrafunnisa & Pullalah, 2000; Soverna et al., 2003; Galati et al., 2006) and a few embryological studies in *Astragalus* (Akhalkatsi et al., 1988; Gvaladze & Akhalkatsi, 1996; Riahi et al., 2003; Riahi & Zarre, 2009) have provided useful characters in embryogenesis and classification. Therefore, in the present study we investigate detailed embryological processes in five *Astragalus* species distributed in Iran including  *A. eriocarpus* (sect. *Malacothrix*), *A.glaucacanthus* (sect. *Poterion*), *A. remotijugus* (sect. *Caprini*), *A. chrysostachys* (sect. *Hymenostegis*) and *A. compactus* (sect. *Rhacophorus*). The main goal of this work is providing valuable characters useful in embryological studies of *Astragalus* which could be applicable in providing synapomorphies and further supports for certain clades known according to former molecular systematic approaches.

# MATERIALS AND METHODS

Buds, flowers and fruits samples were collected at different stages of development in May 2009 and 2010 from Alborz Mt., N Tehran, i.e. Jajrood and Roodehen, Iran. Then samples fixed in FAA, stored in 70% ethanol, embedded in paraffin and sectioned at 6-8 μm with LEICA RM2255 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

The overall megagametophyte and embryo development in *A. eriocarpus*, *A. glaucacanthus*, *A. remotijugus*, *A. chrysostachys* and *A. compactus* is discussed below for all of five species, and the differences are emphasized.

## Ovary and Megagametophyte

The ovules in the five species are arranged in two rows. The mature ovules are campylotropous, crassinucellar and bitegmic. The micropyle is formed by two integuments and is zig-zag in shape (Fig. 1 A, B and Fig. 2 A, B). The embryo sac consists of seven cells: the egg cell, two synergids, the central cell and three antipodal cells (Fig. 1 C, E). Such an embryo sac represents the *Polygonum* type. The degeneration of antipodal cells is the first detectable change in the embryo sac before fertilization (Fig. 1 D). An endothelium is originated from the inner layer of the inner integument. The cells of this layer become radially stretched and contain prominent nuclei and dense cytoplasm. These cells are cubic in shape (Fig. 1 A, B, C, E, F, G, H).

## Zygote Stage

The micropylar part of the zygote is filled by a large vacuole and the chalazal end with cytoplasm containing the egg nucleus. The zygote undergoes a period of rest, or perhaps reorganization. After a relatively long period of dormancy the zygote begins to elongate along the axis of the embryo sac (Fig. 1 F). Unlike zygote, the primary endosperm nucleus does not require any resting period and undergoes divisions within hours after fertilization. Repeated free nuclear divisions produce a large number of nuclei (Fig. 1 G). The embryo sac obtains its maximal growth rate at this stage and become open U-shaped. Also at this stage, in the basal part of the nucellus, between the embryo sac and vascular bundles, there is a group of cells with lignified and suberized walls, which is called hypostase (Fig. 1 F and Fig. 2 A, B). These cells usually have poor cytoplasmic contents and deformed nuclei, but occasionally they may have dense cytoplasm and thin walls like glandular cells (Fig. 2 F). The endothelium keeps pace with the expanding embryo sac by undergoing numerous anticlinal divisions (Fig. 2 B, D, F).
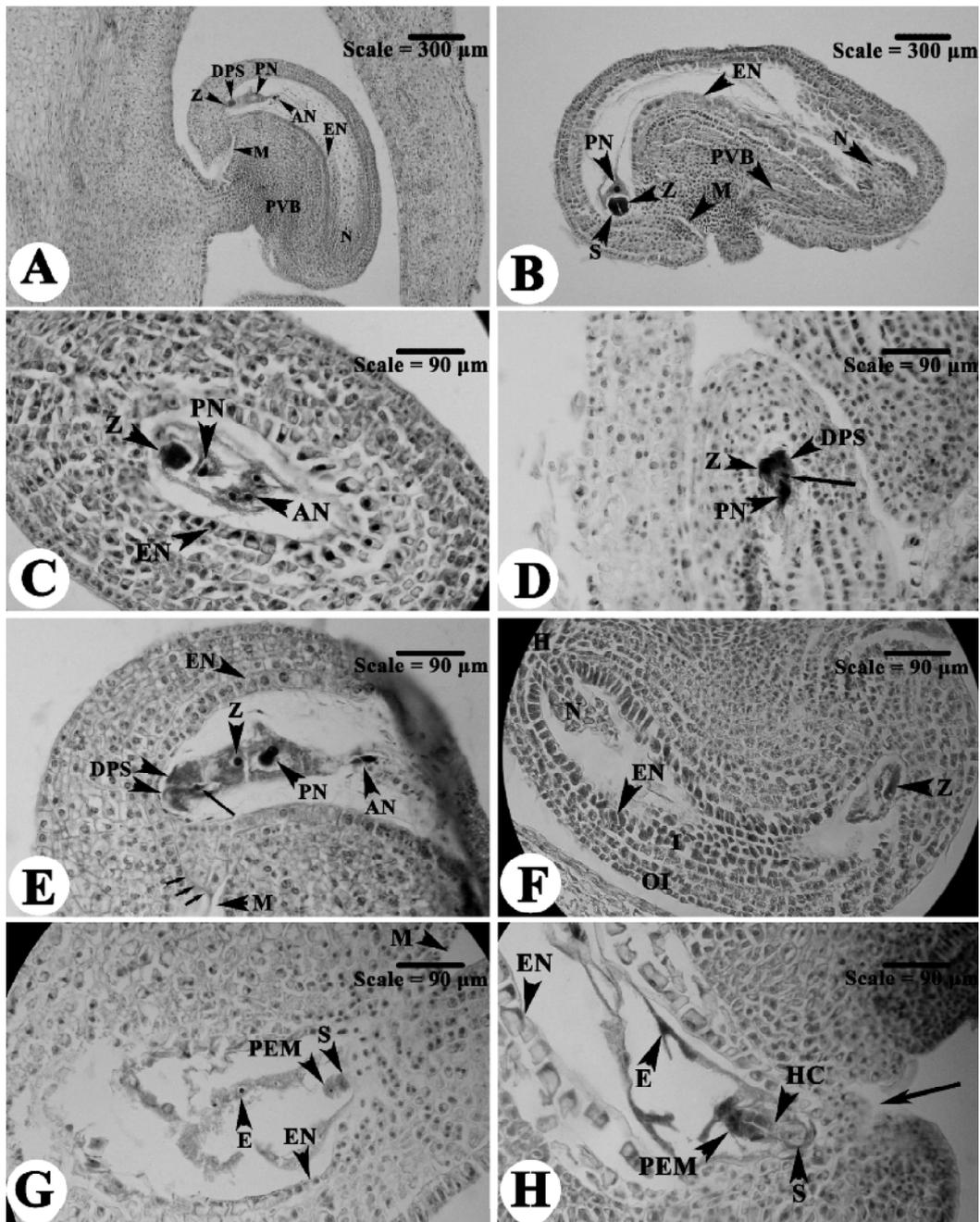
Fig. 1. Megagametophyte organization to proembryo stage. **A.** Longitudinal section of a fertilized ovule of *A. remotijugus*: Megagametophyte including antipodal cells, polar nuclei and zygote. **B.** Longitudinal section of a fertilized ovule in *A. chrysostachys*. **C.** Longitudinal section of organized megagametophyte in *A. glaucacanthus*: Antipodal cells are arranged side by side. **D.** Fertilized ovule in *A. compactus*. **E.** Young ovule just at fertilization in *A. remotijugus*: Fusion of one sperm (arrow) with polar nuclei at middle of megagametophyte. Small arrows show the pollen tube grows toward embryo sac and enters in the micropyle. **F.** Longitudinal section of ovule in *A. eriocarpus*: Zygote begins to elongate. **G.** *A. compactus*: First division in zygote that resulting in a terminal and basal cell and nuclear divisions in endosperm. **H.** Proembryo stage (four cellular embryo) in *A. glaucacanthus*: First divisions of primary endosperm nucleus, arrow show micropyl. **Abbreviations:** An-antipadal cells; DPS- degenerative persistent synergid; EN- endothelium; H- hypostase; HC- hypophyse; I- inner integument; M- micropyle; N- nucellus; OI- outer integument; PN- polar nuclei; PVB- provascular bundle; S- suspensor; S- synergid; VB- vascullar bundle; Z- zygote.
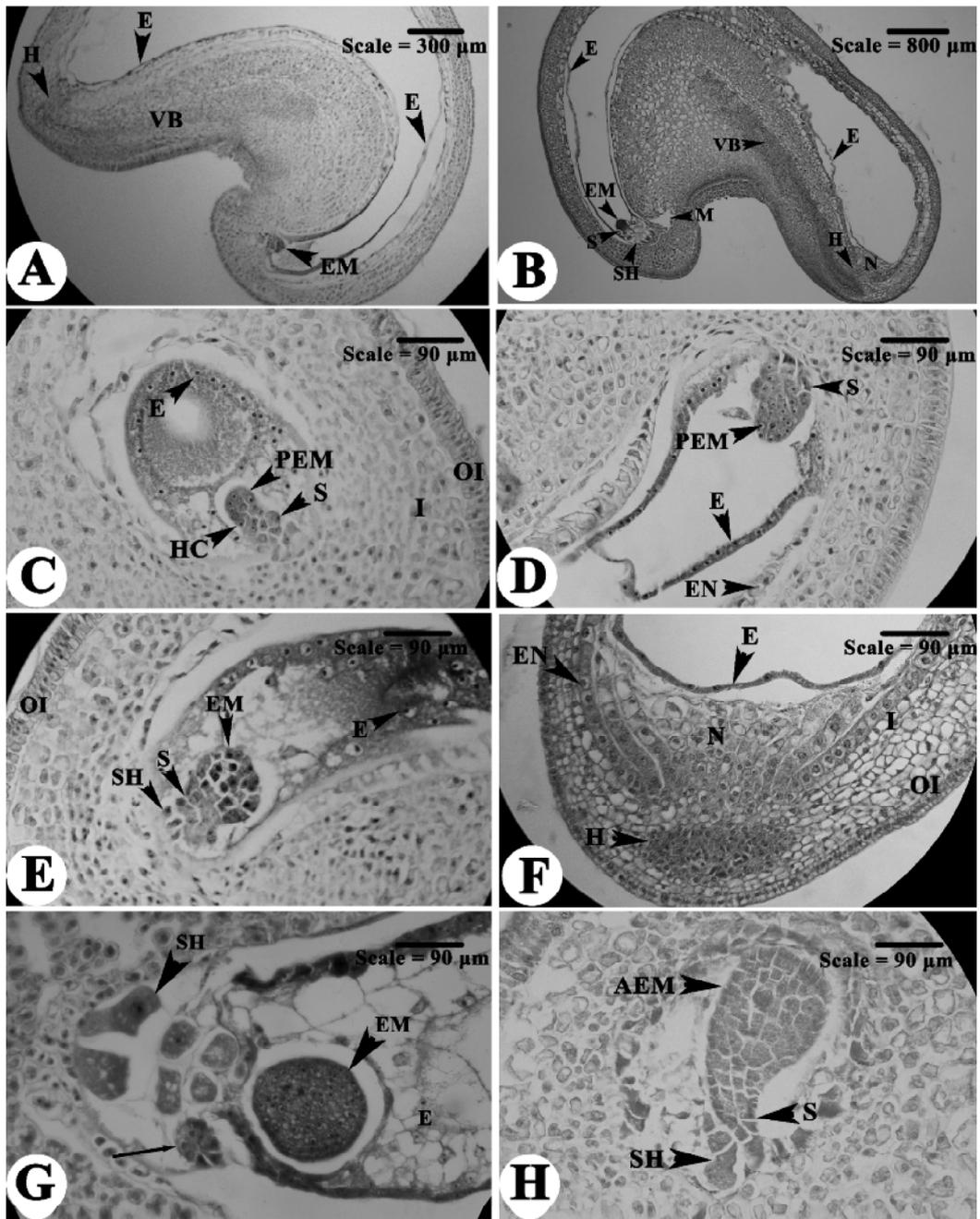
Fig. 2. Development of proembryo to globular stage. A. *A. glaucacanthus*, endosperm develops as peripheral layer around the embryo sac and hypostase tissue is between the embryo sac and vascular bundles. B. Longitudinal section of ovule at proembryo stage in *A. remotijugus*, endosperm is peripheral layer around the embryo sac. C. *A. glaucacanthus*, eight- celled proembryo. D. *A. glaucacanthus*, 16- celled proembryo. E. *A. glaucacanthus*, early globular stage with coenocytic endosperm. F. Hypostase cells in *A. remotijugus*, the basal region of embryo sac that show hypostase, part of nucellus and peripheral endosperm. G. Polyembryony in *A. remotijugus*, lately globular stage with cellular endosperm, arrow show second embryo. H. Abnormal embryo in *A. glaucacanthus* with abnormal suspensor at late globular stage. Abbreviations: AEM- abnormal embryo; E- endosperm; EM- embryo; EN- endothelium; H- hypostase; HC- hypophyse; I- inner integument; M- micropyle; N- nucellus; OI- outer integument; PEM- proembryo; S- suspensor; SH- haustorial suspensor; VB- vascular bundle.

## Proembryo Stage

The embryo development follows the Onagrad type. There are no fundamental differences in the early stages of development in *Astragalus* embryos among studied species. The zygote divides transversely resulting in a terminal and a basal cell (Fig. 1 G). The basal cell divides by a transverse wall and the apical cell longitudinally. The proembryo thus assumes to take the shape of an inverted T. The two terminal cells formed by the longitudinal division of the apical cell divide by a longitudinal wall at right angles to the first and form a quadrant. Each cell of the quadrant divides by a transverse wall, giving rise to the octant stage (Fig. 1 H). The endosperm develops as a coenocytic tissue around the embryo and suspensor (Fig. 2 C, D), and as peripheral layer around the embryo sac (Fig. 2 A, B).

## Globular Stage

The cells of octant enlarge considerably and undergo priclinal divisions (Fig. 2 D) differentiating an outer dermatogens layer and an inner layer of cells. The globular embryo is isometric (Fig. 2 E). Concomitant with the divisions the proembryo transverse divisions in basal cell, giving rise to an elongated suspensor. The proximal cell of the suspensor is conspicuously enlarged and is considered as haustorium (Maheshwari, 1950; Riahi et al., 2003; Riahi & Zaree, 2009). The cellularization of the endosperm occurs at the late globular stage (Fig. 3 B, C, D). This process starts at the micropylar end but the nuclei at the chalazal end remain free and embedded in a common cytoplasm. This chalazal coenocytic part of the embryo sac grows into a tubular haustorium (Fig. 3 G).

## Heart Stage

Continued cell divisions increase the size of embryo. After formation of cotyledon primordial the embryo takes its heart-shaped form (Fig. 3 A, E, F). The main part of endosperm is cellularized at early heart-stage and endosperm haustoria is formed. The outer integument layer form the malpighian cells (macrosclereids) characteristic for the testa of *Fabaceae* (Fig. 3 D, E, F).

## Cotyledon Stage

The two cotyledons begin to grow in to the cellularized endosperm. The cotyledons and hypocotyls elongate chiefly by transverse divisions of their constituent cells. The shoot apex differentiates as a small region in the depression between the two cotyledons (Fig. 3 G, H).

## DISCUSSION

There are several morphological and developmental characters of the ovule in *Astragalus* species which have proved of taxonomic significance. Among the *Astragalus* species studied, two characters consist of: differences in suspensor and the timing of polar nuclei fusion have been marked (Lersten, 1983; Prakash, 1987; Akhalkatsi et al., 1988; Gvalade & Akhalkatsi, 1996; Riahi et al., 2003; Riahi & Zarre, 2009). The present work represents a detailed embryological study of five species belonging to five different sections in the classical systematic of the *Astragalus* genus (Bunge, 1868; Podlech, 1982). Also this paper focuses on important characters of the comparative embryology of five *Astragalus* species and examines the impacts of recent advances in phylogenetic.

## Development of the suspensor

The size and appearance of the suspensor differ greatly among the *Fabaceae* (Lersten, 1983). Also one of the main differences in embryo development of *Astragalus* species is reported to be regarding the suspensor characters (Maheshwari, 1950; Riahi et al., 2003; Riahi & Zarre, 2009). In the investigated species the suspensor in *A. eriocarpus* (sect. *Malacothrix*) and *A. glaucacanthus* (sect. *Poterion*) is composed of four columns of the cells as well as 8-10 haustorial cells. This type of suspensor is smaller than the embryo proper in the globular stage (Fig. 3 A, B). The same suspensor structure has been observed in *A. cemerinus* (sect. *Microphysa*) and *A. ruscifolius* (sect. *Dissitiflori*) (Riahi & Zarre, 2009). Based on molecular data, since these four unrelated species belonging to different clades of *Astragalus* (Kazempour Osaloo et al., 2003, 2005) show this type of suspensor obviously represents the most common one, and probably a plesiomorphic character for the genus. Another type of suspensor in *A. remotijugus* (sect. *Caprini*) is composed of six columns of cells with 10 to 15 large haustorial cells. This type of suspensor is as large as or larger than the embryo proper (about 2.5 times) at the globular stage (Fig. 3 C). Based on present study, comparing with molecular data (Kazempour Osaloo et al., 2003) it seems that in primitive groups of species such as members of sect. *Caprini* species, suspensor would be larger with more columnar cells and large haustorial cells. Another characteristic is observed in this specie, is formation of few embryos which originated from cells of embryo sac other than the egg cell. According to the former studies, commonly the additional embryos develop from synergids (Akhalkatsi et al., 1988; Singh et al., 2001). Here in *A. remotijugus* seem additional embryo is develop from the haustorial suspensor cells of the
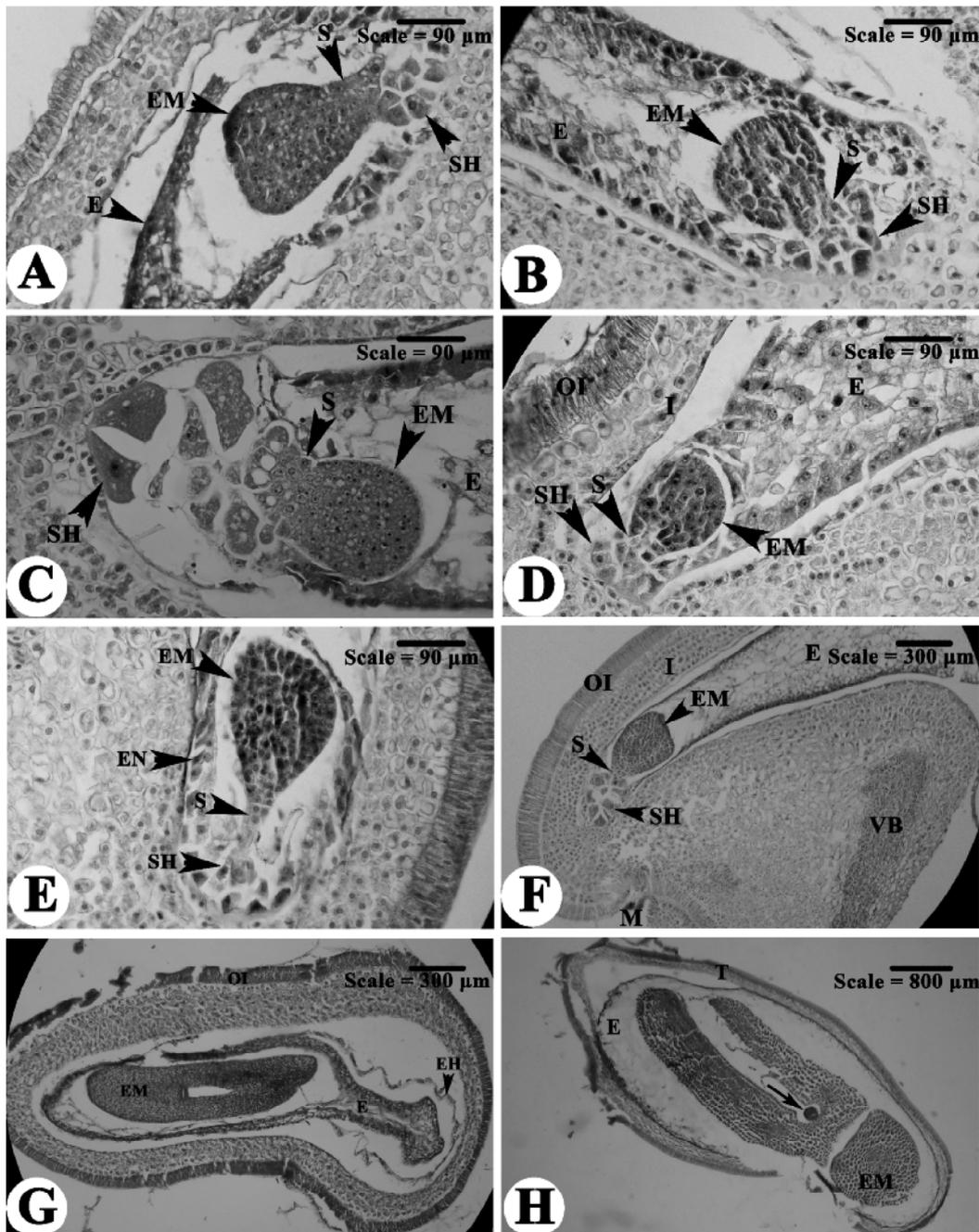
Fig. 3. Comparative size and shape of the suspensor between five studied species. A. *A. eriocarpus* at early heart stage. B. *A. glaucacanthus* at globular stage. C. *A. remotijugus* at globular stage. D. *A. chrysostachys* at globular stage. E. *A. compactus* at early heart stage. F. Heart stage at *A. remotijugus*, the outer integument shows the characteristic morphology of the mature testa and reaches its maximal width during this stage and endosperm is cellularized. G. Cotyledon stage in *A. eriocarpus*, cotyledons growing and endosperm begins to degenerate. H. Cotyledon stage in *A. eriocarpus*, mature embryo with dome shaped apical shoot meristem (arrow). Abbreviations. E- endosperm; EH- haustorial endosperm; EM- embryo; EN- endothelium; I- inner integument; M- micropyle; OI- outer integument; S- suspensor; SH- haustorial suspensor; T- testa.

proembryo (Fig. 2 G). The smallest suspensor was observed in *A.chrysostachys* (sect. *Hymenostegis*) and has three columns of cells which lie in one or two rows with 4 to 6 haustorial cells (Fig. 3 D). According to the molecular systematic studies *A. glaucacanthus* and *A.chrysostachys* belong to same clade of *Astragalus* (Kazempour Osaloo et al., 2003). A three clumns of cells with 3 haustoria cells form the suspensor of *A. compactus* (sect. *Racophorus*) (Fig. 3 E). The same suspensor structure has been observed also in *A. demavendicus* and *A. latifolius* (sect. *Incani*) (Riahi et al., 2003), two columns of cells with 2-3 haustorial cells. These species belong to different clades of *Astragalus* (Kazempour Osaloo et al., 2003).

## Timing of fusion of polar nuclei
According to the last studies, the polar nuclei in *A. latifolius* fuse immediately after fertilization, but in *A. demavendicus* they remain separated until fertilization (Riahi et al., 2003). Then, in *A. cemerinus* and *A. ruscifolius* reported, fusion of polar nuclei occurs in the median regions of the central cell and before fertilization (Riahi & Zarre, 2009). In current study, all of species represented polar nuclei fusion before fertilization in the median central cell (Fig. 1 B, C, D), but in *A. remotijugus* they remain separated until fertilization (Fig. 1 E).

## Digestion of nucellus
The nucellus begins to degenerate after fertilization in *A. glaucacanthus*, *A. eriocarpus A. chrysostachys* and *A. compactus*. In a way in this species when the embryo sac is formed the nucellus is completely degenerated (Fig. 1 A, B, F and Fig. 2 A). But in *A. remotijugus* parts of the nucellus is remained until proembryo stage (Fig. 2 B). The same of this result is obtained in some other *Fabaceae* such as *Vicia faba* L. (Johansson & Walles, 1993). Riahi et al. (2003) observed that the nucellus degenerated when the embryo sac is formed and the degenaration of nucellus is completed until the formation of the proembryo.

## Endosperm development
The development of endosperm in all of five species is similar with other species in *Astragalus* (Akhalkatsi et al., 1988; Gvalade & Akhalkatsi, 1996; Riahi et al., 2003; Riahi & Zarre, 2009). The first divisions of the primary endosperm nucleus are not accompanied by cell wall formation and nuclei remain free in the cytoplasm of the embryo sac. In proembryo stage endosperm is nuclear (Fig. 1 G, H and Fig. 2 A, B, C, D, E). The wall formation in nuclear endosperm begins at the lately globular stage (Fig. 3 A, B, C, D, E, F). In

*Fabaceae* endosperm haustorium developed at the chalazal end (Johri & Gorg, 1959). The nuclei at this part are free and embedded in a common cytoplasm. The endosperm haustorium is observed in all five investigated species (Fig. 3 G).

## Seed abortion
The number of ovules in an ovary and seeds in a pod are 11 and 2 to 4 in *A. eriocarpus*, 10 and 2 to 4 in *A. glaucacanthus*, 24 to 27 and 8 to 12 in *A. remotijugus*, 8 and 1 in *A. chrysostachys* and 4 to 6 and 1 in *A.compactus*, respectively. According to the results in *Pongamia pinnata* (*Fabaceae*), reduced seed set could be a result of competition for maternal resources between the seeds in a pod (Arathi et al., 1999). The last studies about *Astragalus* abnormal embryos were reported in *A. cemerinus* and *A. ruscifolius* (Riahi & Zarre, 2009). Abnormality is accompanied by development of an abnormally curved or obliquely positioned suspensor. In this manner globular embryo cannot advance to the heart shape as it enlarges. A similar kind of abnormality is observed in the five investigated species (Fig. 2 H). However, the mean percentage of seeds with abnormal embryos is about 65% in *A. eriocarpus*, 60% in *A. glaucacanthus*, 55 to 66% in *A. remotijugus*, 87.5% in *A.chrysostachys* and 75 to 83% in *A. compactus*. Therefore the highest rate of seed abortion is in *A. chrysostachys* and the lowest rate of seed abortion is in *A. remotijugus* and *A. glaucacanthus*.

## REFERENCES
Akhalkatsi, M., Beridze, M. & Gvaladze, G. 1988: Embryo and endosperm development in Astragalus caucasicus Pall. -Bulletin of the Georgian National Academy of Sciences 132: 601-604. (in Georgian).

Arathi, H. S., Ganeshaiah, K. N., Uma-Shaanker, R. & Hegde, S. G. 1999: Seed abortion in Pongamia pinnata (Fabaceae). -American Journal of Botany 86(5): 659-662.

Ashrafunnisa, A. & Pullalah, T. 2000: Embryology of Clitoria ternatea (Fabaceae). -Plant Biosystems 134 (1): 39-43.

Bunge, A. 1868: Generis Astragali Species Gerontageae. -Mémoires de l'Académie Impériale des Sciences de Saint Pétersbourg ser. 7, 11(16): 1-140.

Cameron, B. G. & Prakash, N. 1994: Variation of the megagametophyte in the Papilionoideae. In: Ferguson IK, and Tucker S [eds.], Advances in Legume Systematics, 97-115, part 6. -Royal Botanical Gardens, Kew, England.

Galati, B., Rosenfeldt, S. & Tourn, G. 2006: Embryologyical studies in Lotus glaber (Fabaceae). -Annales Botanici Fennici 43: 97-106.

Gvaladze, G. & Akhalkatsi, M. 1996: Comparative embryology of some Astragalus species. -Bulletin of the Georgian National Academy of Sceiences 153: 432-434.

Johannson, M. & Walles, B. 1993: Functional anatomy of the ovule in broad bean (Vicia faba L.), I. Histogenesis prior to and after pollination. -International Journal of Plant Sciences 154: 80-89.

Johri, B. M. & Garg, S. 1959: Development of endosperm haustoria in some Leguminosae. -Phytomorphology. 9: 34-46.

Kazempour-Osaloo, S., Maassoumi, A. A. & Murakami, N. 2003: Molecular systematics of the genus Astragalus L. (Fabaceae): Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacers and chloroplast gene ndhF sequences. -Plant Systematics and Evolution 242: 1-32.

Kazempour-Osaloo, S., Maassoumi, A. A. & Murakami, N. 2005: Molecular systematics of the old world Astragalus (Fabaceae) as inferred from nr DNA ITS sequence data. -Brittonia 57(4): 367-381.

Lersten, N. R. 1983: Suspensors in Leguminosae. -Botanical Review 49: 233-257.

Liston, A. 1992: Variation in the chloroplast genes rpoC1 and rpoC2 of the genus Astragalus (Fabaceae): evidence from restriction site mapping of a PCR amplified fragment. -American Journal of Botany 79: 953-961.

Maassoumi, A. A. 1998: Astragalus in the old world: a Check-List. -Research Institute of Forests and Rangelands, Tehran.

Maheshwari, P. 1950: An Introduction to the Embryology of Angiosperms. -McGraw-Hill, New York.

Palser, B. F. 1975: The bases of angiosperm phylogeny: Embryology. -Annals of the Missouri Botanical Garden 62: 621-646.

Prakash, N. 1987: Embryology of the Leguminosae. In: Stirton CH (ed.), Advances in Legume Systematics, 241-278, Part 3. -Royal Botanical Gardens, Kew, England.

Podlech, D. 1982: Neue Aspekte zur Evolution und Gliederung der Gattung Astragalus L. (Leguminosae). -Mitt. Bot. Staatssamml. München 18: 359-378.

Rembert, D. H. 1969: Comparative megasporogenesis in Papilionaceae. -American Journal of Botany 56 (6): 584-591.

Riahi, M., Zarre, SH., Chehregani, A. & Behboudi, B. 2003: Seed development in two species of medifixed hairy Astragalus (Fabaceae). -Flora 198: 211-219.

Riahi, M. & Zarre, SH. 2009: Seed development in Astragalus cemerinus and A. ruscifolius (Fabaceae), and its systematic implications. -Acta Biologica Cracoviensia Series Botanica 51 (1): 111-117.

Sanderson, M. J. & Doyle, J. J. 1993: Phylogenetic relationships in North American Astragalus (Fabaceae) based on chloroplast DNA restriction site variation. -Systematic botany 18: 395-408.

Singh, V., Pande, P. C. & Jain, D. K. 2001: Embryology of Angiosperms. Part 4: 24-49. -Rastogi Publications, India.

Soverna, A. F., Galati, B. & Hoc, P. 2003: Study of ovule and megagametophyte development in four species of subtribe Phaseolinae (Leguminosae). -Acta Biologica Cracoviensia Series Botanica 45 (2): 63-73.

Wojciechowski, M. F., Sanderson, M. J. & Hu, J. M. 1999: Evidence on the monophyly of Astragalus (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA trnL intron data. -Systematic Botany 24: 409-437.

Zarre, SH. 2000: Systematic Revision of Astragalus L. sect. Adiaspastus, sect. Macrophyllium and sect. Pterophorus (Fabaceae). -Englera 18: 1-219.