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Gametophyte development in *Asperugoprocumbens* (Eritrichieae, Boraginaceae)

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ABSTRACT

Reproductive organs and embryological characters consisting of microsporogenesis, macrosporogensis,andgametophyte developmental process were studied in *Asperugoprocumbens*. The results showed that anther is tetrasporangiate and consisting of four-layered wall with dicotyledonous type development. The middle layer and tapetumweredisappeared during development. Tapetum is combination of secretory and plasmoidal type. Tetrahedral tetrads with simultaneous cytokinesis were produced. Pollen grains were pear shaped, 2-celled and 6-colporate at the shedding stage. The ovule is unitegmic, anacampylotropous, tenuinucellate, funicular and embryo sac development is of *Polygonum* type. A single archesporial cellwasformed that act as megaspore mother cell. The chalazal or micropylar megaspore of the linear or T-shaped tetrad functions as the functional megaspore and produced gametophyte. The mature embryo sac is consists of an egg apparatus at the micropylar end, a central cell in adjacencies of egg apparatus and five antipodal cells at the chalazal end.

KEYWORDS

Ovule;
Anther;
Pollen;
Embryo;
Asperugoprocumbens;
Boraginaceae;
Eritrichieae.

INTRODUCTION

The medicinal family of the Boraginaceae has about 156 genera and 2500 species (Zhu et al., 1995). The most members of Boraginaceae don't have either eatable or industrial uses, but most of theirs flowers, stems, and leafs have medicinal effect. The Boraginaceae divided to five subfamilies by most recent authors[11,55], consist ofEhretioideae, Cordioideae, Heliotropioideae, Boraginoideae andWellstedtioideae, but there are some controversies about their systematic position as separate family or subfamily of the Boraginaceae[11,22-24,26,27,55,56]. *Asperugo* is belonging to the Eritrichieae tribe and Boraginoideae subfamily. It is an annual herb with small calyx and corolla; corolla is violet, blue or white and tubular; lunette throat appendage[60]. *Asperugoprocumbens* is used as strengthen the nervous system, calmative, and antispasmodic, and for treatment of skin septicity,in Iran's traditional medicine[3,52]. Some medicinal attributes consist ofantibacterial and antioxidant activity and radical scavenging activities, have made it as the subject of researches[1,39].

For a good and comprehensive taxonomy, as well

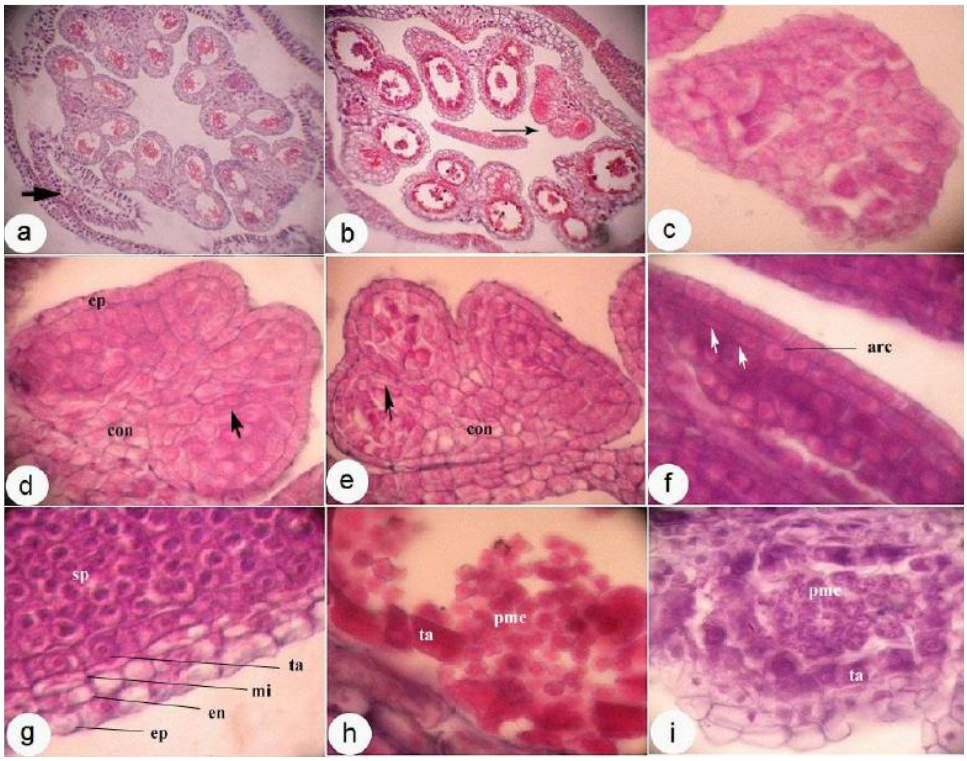

Figure 1 : Anther development, anther wall formation, and microsporogenesis in *Asperugo procumbens*: (a) Transverse section of tetrasporangiate anthers and nectary disc (arrow); (b) the anthers of a flower in various developmental stages; (c) four-lobed anther primordia; (d) differentiate of epidermis, septum (arrow) and connective tissue in anther; (e) difference in developmental stages of two theca attached to an anther; (f) the anther wall formation (arrow) of the archesporial cells; (g) mature anther wall and sporogenous cells; (h) pollen mather cells; (i) prophase I of the pollen mather cells; Abbreviations: ep; epidermis, con; connective tissue, arc; archesporial cell, en; endothecium layer, mi; middle layer, ta; tapetal layer, pmc; pollen mather cells.

as clarifying relationships and phylogeny should use different valuable characters of the taxon. Some embryological characters provide taxonomic important data for the classification[8,37,38,41,48-51,54,58,54]. Based on our bibliographical studies *A. procumbens* was not studied regarding its embryological and developmental characteristics.

MATERIAL AND METHODS

Plant specimens collection area was selected in Broujerd at the west of Iran. The area namely Bishehlocated at the west of Boroujerd city, in Lorestan Province in Iran. In florescence of *A. procumbens*, tiny flower buds and mature flowers were removed in different sizes from area. This flowers that were at various developmental stage were fixed immediately in FAA_{70}(formaldehyde,ethanol, and acetic acid] for the 48 h. Specimens then were dehydrated using increasing ethanol series (30%-100% ethanol), transferred through an ethanol-toluene series (75% ethanol+ 25% toluene, 50% ethanol +50% toluene, 25% ethanol + 75% toluene, 100% toluene twice) and embedded in paraffin wax. The specimens werespliced using of a rotary microtum (DidehSabz, Iran) with 7µm thickness. The samples were stained using haematoxylin-eosin (Chehregani, et al., 2014). The samples were observed by a light photomicroscope (Zeiss AxiostarPluse, Germany)and microphotographs were taken from the best ones using a digital camera (Canon, G_{11}, Japan).

RESULTS

Formation of the anther wall

Results showed that *A. procumbens*has five stamens (Figure 1a) and its anther is tetrasporangiate (Figure 1b). During early developmental stages, anther primordial resulted to formation four-lobed anther (Figure 1c). The outer layer of the anther primordium differen-

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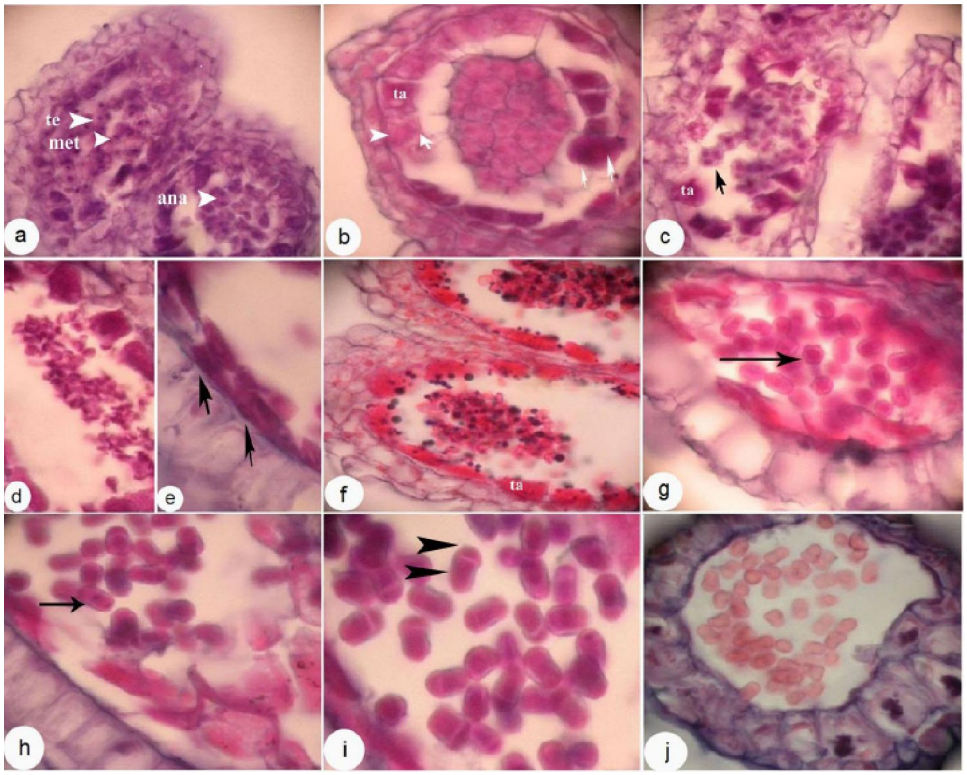

Figure 2 : Microsporogenesis and male gametophyte in *A. procumbens*; (a) metaphase I, anaphase I and telophase I of the microsporocyte; (b) telophase II and two nuclear tapetal cells; (c) tetrahedral tetrad and hybrid tapetal cells; (d) non vacuolated free microspores; (e) two layers tapetal cells; (f) black grains in pollen sacs;(g) hexagonal pollen grain in Transverse section; (h) nuclear division in microspores; (i) 2-celled and elliptical pollen grains before dehiscence; (j) pear shaped pollen grains in dehiscence; Abbreviations: te; telophase I, met; metaphase I, ana; anaphase, I ta; tapetal cells.

tiates to epidermis (Figure 1d), and then microsporangiawere formed by septum differentiation between theanther lobes (Figures 1d-e).

The anthers are located alternatively with petals and have five nectar disks (Figure 1a), as well as several immature stamens are occurring at the base of ovary (Figure 3f). Sometimes, different developmental stages are observed in the stamens of a single flower (Figure 1b). A few developmental differences are observed among microsporangiums of an anther too (Figures 1d, e). At an early stage of development, a row of archesporial cells with prominent nuclei differentiate below the epidermis. These hypodermal cells dividedpericlinically and cause to form an outer primary parietal cells and inner primary sporogenous cells (Figure 1f). The primary parietal cells divide periclinically to form two layers of secondary parietal layer, the inner secondary parietal layer function directly as the tapetum, and the outer secondary parietal layerproduced the endothecium layer and a middle layer by further division. Thus, maximum number of the anther wall layers is composed of four layers: epidermis, endothecium, a middle layers and tapetum (Figure 1g). The layers number of the anther wall is unstable. Soon after formation the anther wall, the middle layer is crushed (Figures 1h, i). Tapetum, in the stage of microsporocyte, has maximum differentiation and showed high density of cytoplasm (Figures 1h, i, 2a). Some tapetal cells become binucleated (Figure 2b) or they are layered due topericlinal divisions (Figure 2e). The tapetal cells degenerated gradually during the pollen development. This process starts at the tetrad stage (Figure 2c) and completed at the stage ofpollengrains maturation (Figures 2j, 3a-e). At the tetrad stage some tapetal cells maintain their location and some other move into the anthers locule (Figure 2c). Sometimes in free microspore stage, the black globular grains at the different sizes are observed in the locule (Figure 2f). When the pollen grains are mature, the anther wall is consisting of: epidermis and endothecium (Figures 2j, 3a, b, c, d, e).

The endothecium elongated radially and developed fibrous thickenings (Figures 2j, 3a- e). The septum be-

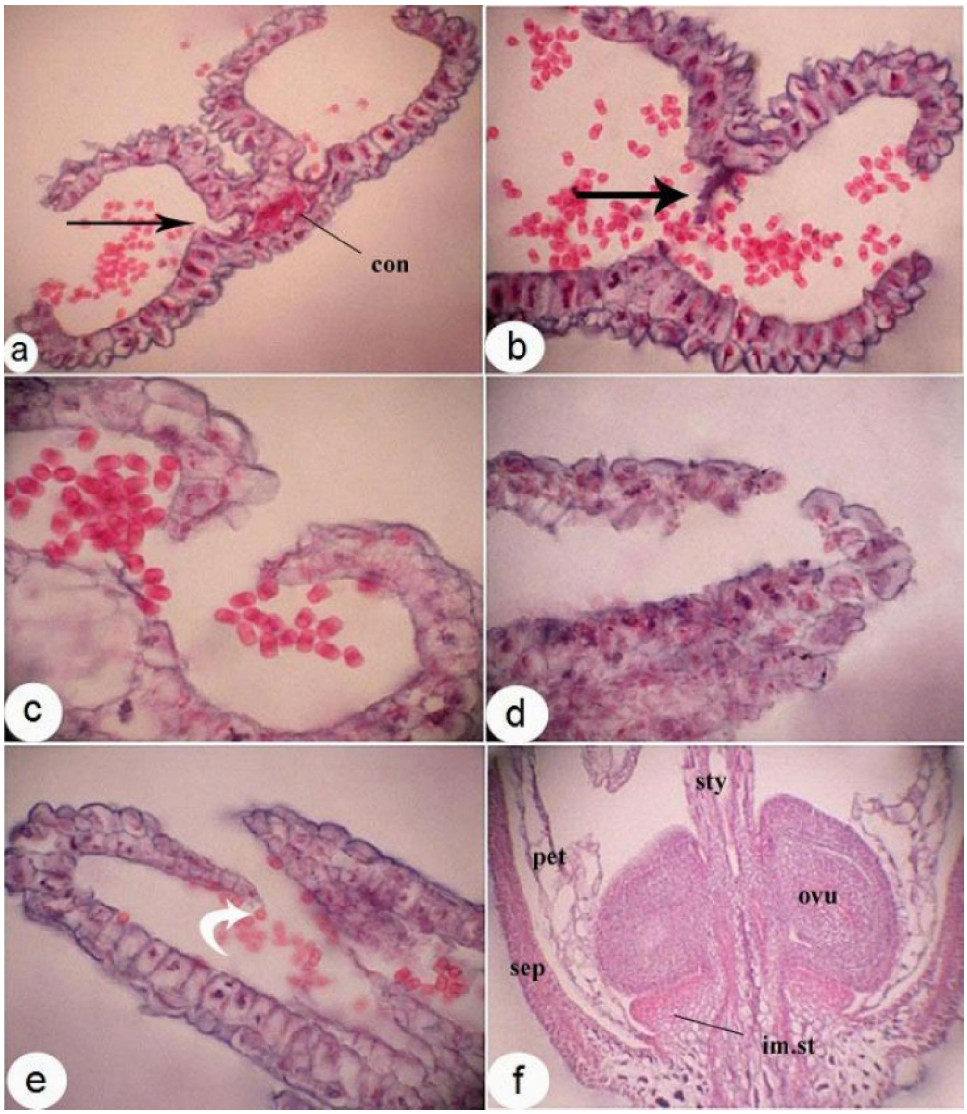

Figure 3 : various dehiscence in anthers of *A. procumbens* and immature stamens: (a) simple dehiscence and starting degenerated of connective tissue (arrow); (b) degenerated of connective tissue completely; (c) dehiscence of stomium; (d) simple dehiscence in corners of anther; (e) invagination of anther wall in corners of anther; (f) immature stamens in base of ovary; Abbreviations: con; connective tissue, sep; sepal, pet; petal, sty; style, ovu; ovule, im.st; immature stamen.

tween the two pollen sacs of a thectumdisappears and the two pollen sacs communicate with each other (Figures 3a-c), as well as connective tissue is disappears (Figures 3a, b). Simple dehiscence of the anther wall is occurredbetween of two pollen sacs or corners of pollen sac (Figures 3a, b, d). The stomium between the two pollen sacs (Figure 3c) and invagination of the anther wall (Figure 3e) dehisce and pollen grains were released.

Microsporogenesis and male gametogenesis

The primary sporogenous layer produced from division of the archesporial cells, divided periclinally and anticlinally, forming secondary sporogenous tissue (Figure 1g) which differentiate into microspore mother cells (PMC). The microspore mother cells are irregular in shape with large nuclei and dense cytoplasm (Figures 1h, i), which are adhesive to each other at beginning of development.

Microsporocytes are separated laterduring Meiosis ⇩ and II, with simultaneous cytokinesis resulted to form tetrahedral microspore tetrads. The microspores are enclosed in a hyaline thick callose wall (Figures 1i, 2a, b, c). Concurrent by maturate microspores, the callose wall become break up and the haploid microspores are released from the tetrad. The microspores

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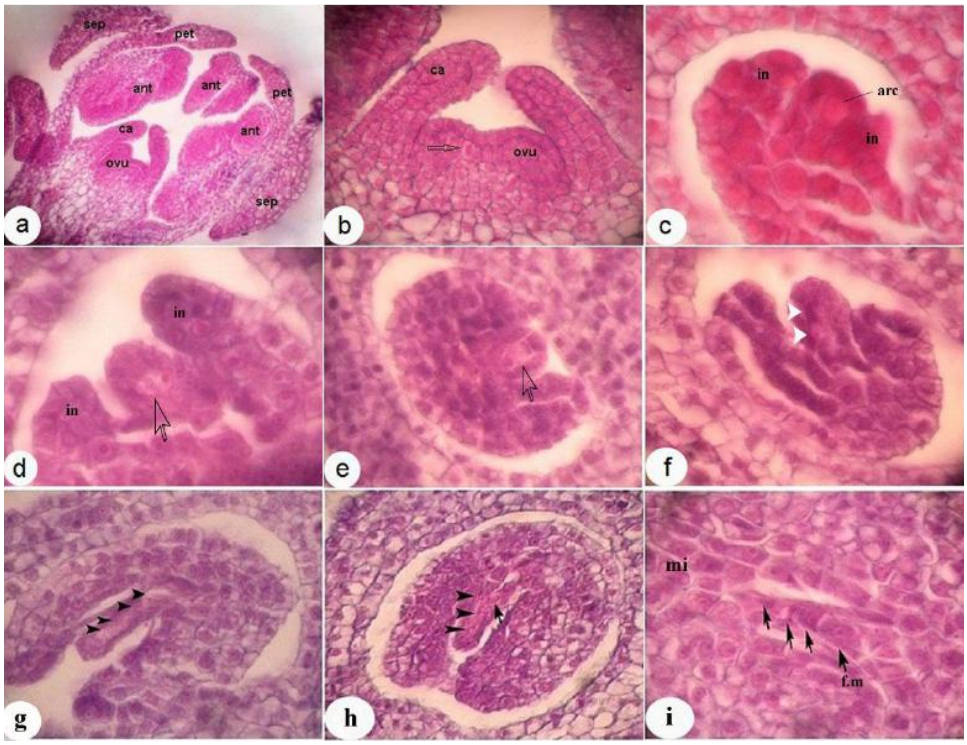

Figure 4 : Flower primordium and megasporogenesis in *A. procumbens*; (a) flower primordium; (b) open carpel in early developmental stage and hypodermal divisions (arrow); (c) archesporial cells and structuralization of integument; (d) the megasporocyte; (e) anaphase I in the megasporocyte; (f) dyad (end of meiosis I) of megaspores; (g) end of meiosis II of the megasporocyte and linear arrangement of the nucleus without cytokinesis; (h) T-shaped megaspores tetrad; (i) degeneration of 3- cell in micropylar end and chalazal functional megaspore; Abbreviations: ca; carpel, sep; sepal, pet; petal, ovu; ovule, ant; anther, in; integument, arc; archesporial cell.

just released from tetrads have no vacuole and they are irregular in shape, with a dense cytoplasm and a centrally placed nucleus (Figure 2d). Subsequently, the microspores become vacuolated and elliptical shape, the nucleus finds coastal location. The mature microspores undergo mitotic division resulting 2-celled pollen grains with high difference in size. The small cell is generative and the other cell is vegetative (Figures 2h, i). Pollen grains are pear shaped atshedding time (Figure 2g-j).

Macrosporogensis

The gynoecium is consists of a single compound pistil consisting of two carpels, a single styleand a superiorovary with 4 locules, each containing a single ovule. The carpelsare open when the first ovular primordium appears and they will close later (Figures 4a, b). In floor of ovary, dome-shaped ovular primordium is formation by periclinal and anticlinal division (Figures 4a, b). The basal-axile ovule is unitegmic, ana-campylotropous, tenuinucellate and funicular (Figures 4c, d, e, 5d, e, f, 6h, k, i). The integument has 10-15 layer of cellsat mature embryo sac stage. Denticulate apophysis is formed from the outer layer of the integument that caused to its conjunction with ovary wall (Figures 6k, l).

A single cell is differentiated in among hypodermal cells of ovular primordium as the archesporial cell (Figure 4c), and then differentiated directly into a megasporocyte (Figure 4d). The megasporocyte is distinguished of the other cells by large nucleus and dense cytoplasm. In stage of archesporium, the integument primordium is appeared at the base of the nucellus (Figure 4c). One layer of the nucellus cells surrounded megasporocyte, thus the nucellus is tenuinucellate. Meiosis and successive or simultaneous cytokinesis of the megasporocyte produces a dyad (Figures 4e, f) and subsequently a linear or T-shaped tetrad of megaspores (Figures 4g, h). The chalazal or micropylar megaspore function as the gametophyte mother cell (the functional megaspore) and the other haploid megaspores become degenerated (Figures 4i, 5a).

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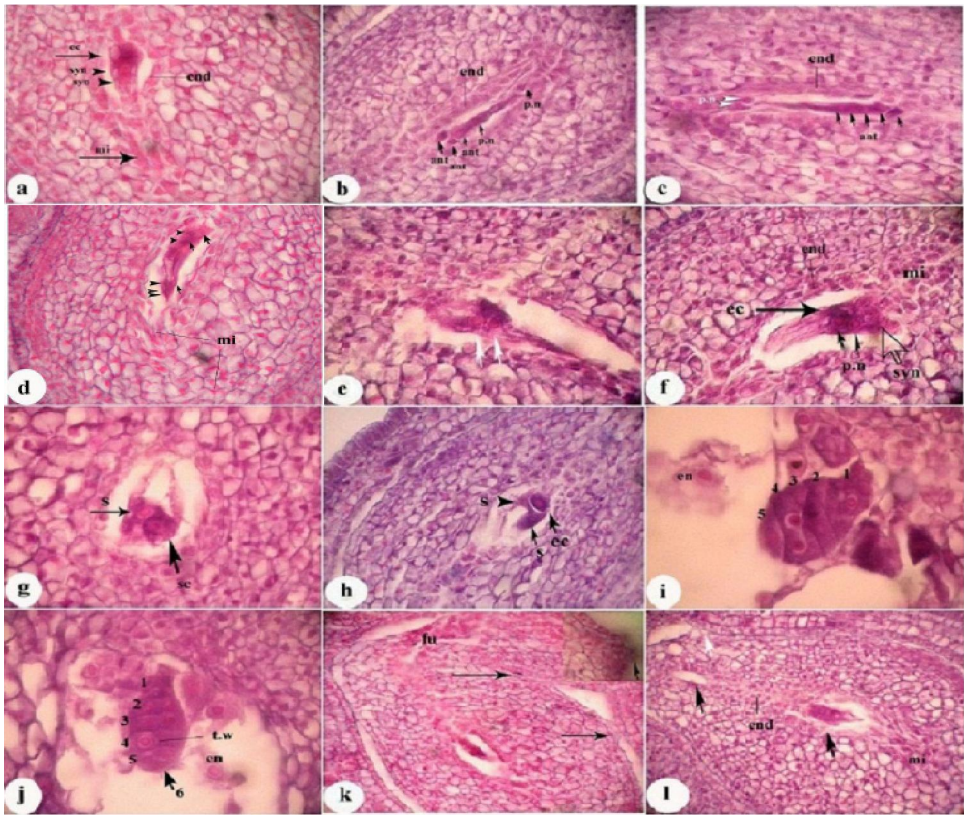

Figure 5 : Megasporogenesis and female gametophyte in *A. procumbens*; (a) degeneration of 3- cells in chalazal end, micropylar functional megaspore; (b) survivor megaspore in depth 3-layer cells; (c) produced survivor megaspore in more depth of cells; (d) 2-nuclear pollen sac and residual nucellus cells (e) 2-nuclear pollen sac in more depth of cell layers and residual nucellus cells, one sided development in integument; (f) 4- nuclear pollen sac and degenerated nucellus cells completely; (g) 4- nuclear pollen sac in much depth; (h) 4- nuclear pollen sac during division for produced 8- nuclear pollen sac, chalazal cells divided before micropylar cells; (i) four cells at the the chalazal end and beginning migration polar nuclei; Abbreviations: f.m; functional megaspore, mic; micropyle, nu; nucellus cell, ec; egg cell, syn; synergid cell, ant; antipodal cell, p.n; polar nuclei, end; endothelium.

Embryo sac and female gametophyte

The 8- nucleatedmegagametophyte by the Polygonum type is formed undergoes three successive mitotic divisions of the functional megaspore. Functional megaspore divided mitotically and produced 2-nucleated embryo sac (Figures 5d, e), then 4-nucleated embryo sac (Figures 5f, g) andfinaly 8-nucleated embryo sac, that four nuclei located in chalazal end and other four nuclei in micropylar end (Figures 5h, i, 5b, d). In 4-nucleated embryo sac, the cells of chalazal end divided priorto the cells of micropylar end (Figure 5h).

One nucleus from the micropylar end and one nucleus from the chalazal end migrated to the center of mature embryo sac to form central cell (Figures 5i, 6b, c, e). The cells located atmicropylar end consisting ofan egg cell, and two synergids that forming the egg apparatus (Figure 6a). The three cells of the chalazal end (Figure 6b) divided to product five antipodal cells (Figure 6c). Polar nuclei migrated towards the egg apparatus and attached to it (Figure 6f). Two polar nuclei later fused and its fertilization by one of the sperms cause to form endosperm cell (Figures 6g, i, j). Egg cell fused by other sperm for production of zygote (Figure 6h). An embryo, withshort suspensor, is produced by successive division of zygote (Figures 6i, j). In various ovules, depth of embryo sacs is different (Figures 5d, e, f, g). Endothelium, with one or two cell layers, originated from the integument that is visible at the around of embryo sac (Figures 6a, b, c, f, l).

DISCUSSION

Earn data of male *A. procumbens* showed that, anther wallis consisting of epidermis, endothecium, a

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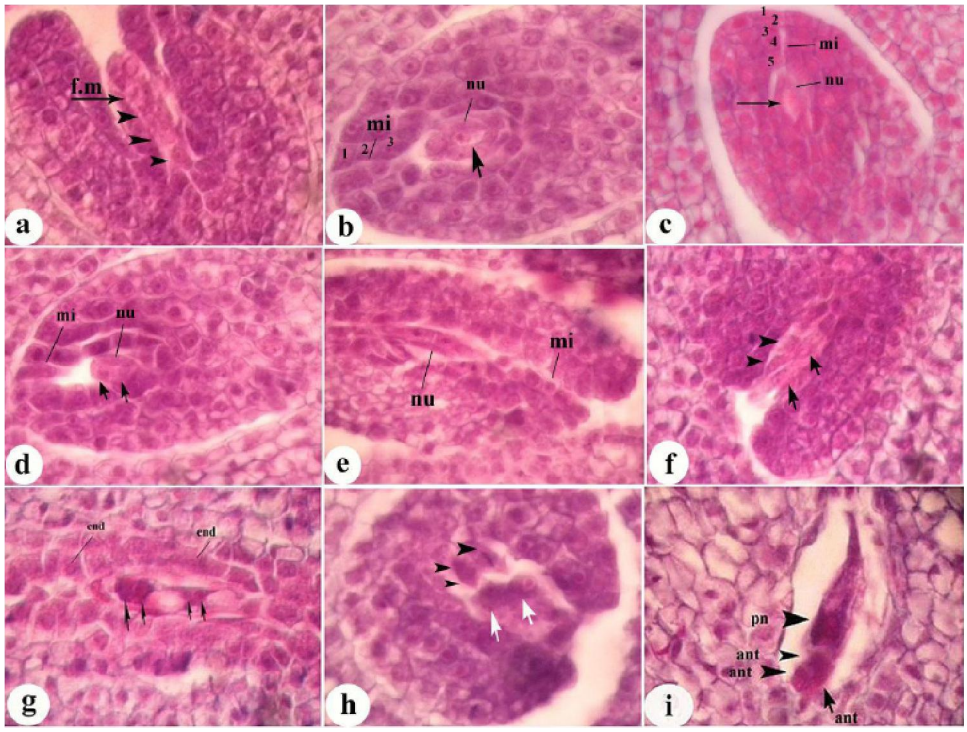

Figure 6 : Female gametophyte, embryo and junction integument and ovary wall in *A. procumbens*; (a) Egg apparatus in composed of two synergids and one egg cell in micropylar end and curvature micropyle, endothelium at the around of embryo sac; (b) three antipodal cells at the chalazal end and two polar nuclei far away each other; (c) duplication of the antipodal cells and two polar nuclei in adjacencies each other; (d) 8- nuclear embryo sac with four cells in chalazal end and four cells in micropylar end, curvature micropyle; (e) junction of polar nucleuses; (f) egg cell, sited polar nucleuses in adjacencies egg apparatus and starting degenerated synergids; (g) sperm in adjacencies of secondary cell (fused polar nucleuses); (h) two sperm in adjacencies of the egg cell; (i) embryo by short suspensor and nucleus endosperm without transverse wall; (j) embryo by transverse wall; (k) junction integument and ovary wall; (l) curvature embryo sac and junction integument and ovary wall; Abbreviations: ant; antipodal cell, syn; synergid cell, p.n; polar nuclei, s; sperm, s.c; secondary cell, en; endosperm, sus; suspensor, em; embryo, fu; funicule; , ec; egg cell, mi; micropyle, end; endothelium, t.w; transverse wall.

middle layer and a tapetum layer. Process of formation anther walls at the different plant is followed four methods suggested, centralist or monocotyledonous[4], eccentricity or dicotyledonous[4,12], basic type and reduced type[12] that *A. procumbens* is followed of dicotyledonous.

Inner layer of anther wall or tapetum layer is single layer in Boraginaceae; generally, its age is longer of middle layer and started to disappear at the microspore tetrad stage. Development of this layer is occurred as secretory type and or amoeboid type at the different plant[42]. Recently, at the *Symphytumofficinale*[16], *Cichoriumintybus*[10], *Psilotumnudum* and *Schizaeapectinata*[45,46] a different type of tapetum is resulted. In these plants, bound of secretory and amoeboid type is broken, and tapetum cells are both secretory and amoeboid type. Some of tapetal cells move to center loculus and other cells maintain their location. Behavior of tapetum in *A. procumbens* indicates to hybrid of secretory and amoeboid too. In free microspore stage, black grains are observed in locule, vicinal of microspores. It is maybe that they are starch grains released of tapetal cells during degenerated for feed of microspores.

In *A. procumbens*tapetal cells are uninucleate in early developmental stage but some of them become binucleate at the later stage, and rarely, single layer tapetal cells become bilayer in some locations, as in *Ehretiaovalifolia, E. microphylla and E. laevis*[19]. In Boraginaceae, both uninucleate[30,31,40] and binucleate[34,43,59] tapetal cells are reported too.[20] in their report on 9 species of Boraginaceae among *A. procumbens* expressed that in investigated species tapetum cells are uninucleate initially become two or three nucleate or uninucleate and polyploidy in later stage, that is not according by our result.

Unlike of other Boraginaceae[32,34,35] greatdiversity in form and location dehiscence of the pollen sacs is observed in *A. procumbens*. In mature anthers, the septum between the two pollen sacs of a theca disappears and the two pollen sacs coalesce before dehiscence. The stomium is dehisces between the two pollen sacs, as well as simple dehiscence and invagination of anther wall is occur between the two pollen sacs and in adjacence connective tissue in corner of pollen sacs. The connective tissue at the *A. procumbens* is degenerated during maturation of anther and dehiscence. Invagination of anther wall, dehiscence of anther wall in adjacent connective tissue and degenerated the connective tissue are not reported in Boraginaceae heretofore.

Process of microsporogenesis in the micro sporangiums of an anther and also anthers of a stamen is not simultaneous completely; sometimes one of the five anthers passed developmental stages very later of other anthers. Also immature stamens are observed at base of the ovary, results of that not reported in Boraginaceae embryology as yet. The massive sporogenous tissue during more differentiation resulted to pollen mother cells. Pollen mother cells in *A. procumbens*undergoes meiosis and simultaneous cytokinesis results also in a tetrahedral microspore tetrad that surrounded by thick callose cover. Tetrahedral tetrad is usual shape of microspores tetrad in plants.[20] reported isobilateral tetrads in their observation for *A. procumbens* that is against our result.

Pollen grains in reported species of Boraginaceae are elliptical, spherical or dumb-bell shaped[20,21,28,31,34,43,53]. In *A. procumbens*, microspores released of tetrad are non-vacuolated and irregular in shape that with vacuolated, they become elliptical. Nucleus of elliptical microspores divided and 2-celled pollen grains are produced, in sheding, pear shaped pollen grains are released, whereas exine thickens is increased in pollen grains and cells are not observable. This result is not accordance with the prior findings for this species[20] and it is initial report pear shaped for Boraginaceae too. Report of[20] indicted to3-celled pollen grains of *A. procumbens* in time of dehiscence that is not accordance by our result. But we opinion that, its maybe that pear shaped pollen grains are as 3-celled pollen grains in time of sheding.

Hexagonal pollen grains in transverse section are observed in *A. procumbens* that its sides indicate to 6 colporate. *A. procumbens* is Heterocolpateand consist of 3 true apertures alternating with 3 pseudoapertures[13]; all of the Eritrichieae are heterocolpate[2,13,21,47]. Our studies showed that colporates in longitudinal section caused to a medial, equatorial constriction that is in accordance with the findings of[21]. Pollen grains by medial, equatorial constriction is observed in many of Boraginoideae.

Starting of ovule development is at the inner morphological surface of the carpels, on the placentae[14,15]. Ovule primordium in *A. procumbens* by hypodermal divisions is formationon the placentae (Figure, when the carpel similar to their primaryancestor, gymnosperms, is open. Species of plant genetic contents will determine physical structure of ovule during development. Micropyle, funicule, structures of ovule and its location at the ovary are taxonomically important characters at the flowering plants.

Shape of the ovule in present research, as a taxonomically important character, based on ovule classifications of[5], ana-campylotropous suggested, although hemianatropous ovule is reported by[20] for this taxon. However, we observation little curvature in ovule (Figures 6h, k), one sided development in integument (Figures 5e, f) and curvature in embryo sac and micropyle (Figures 6a, b, c, d, l) too that caused to our result for add campylotropous. The ana-campylotropous ovule is related to unequal growth at the funicular region and one sided development of integuments and nucellus[5-7,9,17].

One other important character in ovule is funicule that hasdifferent length at the different ovules.[36] recognized that ovules are stalks or sessile. Sessile ovule may have a narrow or an extensive attachment region[15]. At *A. procumbens* funicular ovule is observed. Funicule is short and its unequal growth caused to dangle of ovule.

On base of categorized integument by[57], *A. procumbens* is unitegmic similar to other Boraginaceae members[29]. The first integument of *A. procumbens* is appeared in archesporial stage. Prior researches on *A. procumbens* showed that the primordial initial of ovule is by dermal priclinal division at the archesporial stage[20]. During ovule maturity, some of acrotic cells of integument are jagged then adjoined to inner surface of

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ovary. It's maybe an incompatible character that caused to adjoined seed and ovary as to hard separate seed from the ovary and finally its unfavorable scatter in environment. Our report is the first report of adjoined ovule integument and ovary, on base of our knowlege.

Amount of the nucellus is important in seed scatter in environment. Periclinal division in archesporial cells caused to increase of nucellus layers number and produced crassinucellar ovule, whereas function archespor directly as megasporocyte caused to tenuinucellate ovule. Nucellus in Boraginaceae is heterogeneous and both of crassinucellar, in *Echiumvulgare*L.[25], *Cordiasebestena*[24], and tenuinucellate, in *Ehretiaovalifolia, E. microphylla, E. laevis*[19], *Heliotropiumscabrum, H. strigosum*[34], 9 species of[20] and as *A. procumbens*, ovule at the Boraginaceae are reported. Tenuinucellate ovule is advanced to crassinucellar ovule because the seeds are weightless and they are better scattered in environment. A layer of nucellus enveloped megasporocyte in *A. procumbens*, which it's absorbed during maturation of embryo sac. Remnants of the nucellus are visible in the 2-nucleate embryo sac but in 4-nucleate embryo sac are degenerated completely.

One row of the inner epidermis in integument differentiated as regular cells and surrounds embryo sac of *A. procumbens*. These cells are integumentary tapetum or endothelium that by periclinal divisions resulted to 2-layers endothelium at the some regions of this layer. Commonly found in sympetalous plants with unitegmic and tenuinucellate ovules, the integumentary tapetum exhibits great diversity in its distribution, morphology, cytology, differentiation, and behavior[29]. Endothelium is not reported in embryology studies of Boraginaceae members based on our knowledge as yet.

In Boraginaceae species by monosporic (*Polygonum*type), bisporic (*Allium* type) and both monosporic and bisporic (*Polygonum*and *Allium* type) development of gametophyte is reported[18-20,25,28,31,33,35,53]. Results of this research showed that, development of embryo sac in agreement with observation reported by[20] represent the *Polygonum*type[38] or Normal type[44] as described for more than 80% of angiosperms. Embryo sac in this species is result of meiosis in megasporocyte and thenmitotic divisions in the chalazal or micropylar megaspore at the linear or T-shaped tetrads that the micropylar functional megaspore is not in reports of[20]. Generally, successive cytokinesis is occurred during meiosis in megasporocyte as in *A. procumbes* based on Figures of prior reports[20], but we observation the megaspores tetrads in *A. procumbens* that cytokinesis is simultaneous too. With comparison of several embryo sacs, we resulted that different depths of embryo sac is observation in *A. procumbens* (Figures 5d by 5e & 5f by 5g). It is seemed thatdepth of embryo sac as length of micropyle is dependent to location of functional megaspore, and in the micropylar functional megaspore is under the chalazal functional megaspore. Micropyle in the micropylar functional megaspore is formation by forerunner surface of the integument. In the chalazal functional megaspore, lateral surface of the integument assisted by forerunner surface for produced micropyle that caused to increase micropyle length. The micropyle length in arrive sperm to egg cell is important and make to our opinion that micropylar functional megaspore is advanced character to the chalazal functional megaspore because micropyle is shorter.

Early production of megagametophyte in *A. procumbens* is 2- synergids cell, one egg cell, a central cell and 3- antipodal cells. In following, Synergids cells sited in linear arrangement with egg cell, they are starting degenerated before of fertilization (Figure 6f). Antipodal cells during mitotic divisions result to 5 cells in linear arrangement (Figure 6c), which is not reported by[20]. Addition antipodal cells numbers is in accordance with the findings of[53] on *Heliotropiumeuropaeum*. Egg cell division resulted to an embryo by short suspensor. Form of embryogeny in *A. procumbens* is an unusual form in Boraginaceae. In this species, zygote divides by vertical wall and resulted to binuclear and pantaseriateemberyo, whereas organization of nucleuses is vertical in first layer (suspensor), but in other layers is horizontal (Figure 6i). With apical cell division and produced hexaseriate embryo, transverse wall is formation in between of horizontal nucleuses and vertical wall between nucleuses of first layer (Figure 6j). Unlike of *A. procumbens*, in *Heliotropiumscabrum* (khaleel, 1978), *Ehretiamicrophylla and E. laevis*[19], basal and terminal cells are formation by zygote division and then terminal cell divided with transverse wall.

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