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Microsporogenesis, megasporogenesis and gametophyte development in *Senecio glaucus* L.

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Abstract: Sporogenesis and gametophyte development in *Senecio glaucus* were studied in this study. Flowers and buds in different developmental stages were removed, fixed in FAA_{70}, stored in 70% ethanol, embedded in paraffin and sectioned at 7-10 µm with a microtome. Staining was carried out with PAS and contrasted with Hematoxylin. The results showed that anthers are tetrasporangiate and anther tapetum is of the amoeboid type. Microspore tetrads are tetrahedral and isobilatral. Pollen grains are bicellular at shedding time. The most of microspores (about 60%) are large in size and vacuolated considerably. They degenerated in the late stages of anther development but smaller ones are functional microspores. Ovule is anatropous, unitegmic and tenuinucellate. A 7-celled embryo sac is formed corresponding to the *Polygonum* type. The shape of megaspore tetrads is both tetrahedral and T-form. Functional megaspore is the chalazal one. Embryo sac is very small at the beginning of development, so that its nuclei are arranged as linear, but later its size increases. Antipodal cells are perennial and their number increases up to 8-14. Their nuclei become polyploid. They were also able to form an embryo at the chalazal pole in about 30% of florets, which detects gametophytic apomixis in this taxon. *S. glaucus* may be considered facutatively apomictic species with predominating sexual reproduction.

Keywords: Asteraceae, microsporogenesis, megasporogenesis, agamospermy, *Senecio glaucus*.

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Introduction

Asteraceae is the largest plant family. The family comprises more than 1600 genera and 23000 species (KADEREIT & JEFFERY 2007; FUNK et al. 2009). Its many genera and species, its worldwide distribution and the fact that it comprises many useful plants have made it the subject of many karyological studies (WATANABE 2002; MARTIN et al. 2009; CHEHREGANI & MEHANFAR 2008; CHEHREGANI & HAJISADEGHIAN 2009; ATRI et al. 2009; CHEHREGANI et al. 2011; 2013). The circumscription of the genera is often problematic and some of these have been frequently divided into minor subgroups (HIND et al. 1995; DINÇ & BAĞCI 2009). The principal taxonomic problems within the family are interrelationships amongst the genera, the circumscription of sub-tribal taxa and polymorphic species (TORREL et al. 1999; INCEER & BEYAZOGLU 2004; CHEHREGANI & MEHANFAR 2007, ARABACI & YILDIZ 2009).

Essential oils, secondary metabolites and medicinally important compounds with or without bioactivity, have been isolated from Asteracean species (EARLE et al. 1964; BEERENTRUP & ROBBELEN 1987; HEYWOOD & HUMPHRIES 1997). Many karyological and cytological studies of this taxon have been done (VALLES et al. 2005). Nevertheless, it seems that there are few developmental studies, so new studies are still necessary to improve the knowledge of these plants (VALLES, et al. 2005). Based on present developmental embryological studies, many exceptional events were reported in the members of this family, including Nemec phenomenon (DAVIS 1968; BATYGINA 1987), increasing of synergids (CICHAN and PALSER 1982), increasing of antipodal cells (RICHARDS 1997; PANDEY 2001), four-celled female gametophyte (HARLING 1951) and apomixis (DAVIS 1968; CHAUDHURY et al. 2001). The study is aimed at the study of sporogenesis and gametophyte development in *Senecio glaucus.* Although there are some reports about other species of *Senecio* (RANGASWAMY & PULLAIAH 1986; LAKSHMI & PULLAIAH 1979; HISCOCK, et al. 2003), this is the first embryological investigation in *Senecio glaucus.*

Material and Methods

The *Senecio glaucus* L. plants used in this study were collected from natural population in Hamedan. Voucher specimens are deposited in the herbarium of the Bu-Ali Sina University (HBAS). The voucher specimen is (BUH 10998), labeled as follows: Iran, Hamedan province, 15 km from Hamedan to Bahar, Barfjin village, Alt. 2500 m. The florescence and buds, in different developmental stages, were removed, fixed in FAA_{70} (formalin, glacial acetic acid and 70% ethanol, 5:5:90 v/v), stored in 70% ethanol, embedded in paraffin and sectioned at 7-10 µm with a Micro DC 4055 (Dideh-Sabz Co., Iran) microtome. Staining was carried out with PAS (Periodic Acid Schiff) according to the protocol suggested by YEUNG (1984) and contrasted with Meyer's Hematoxylin. For each developmental stage, several sections were studied under a Zeiss Axiostar Plus light microscope. At least 20 flowers were studied for each stage and photomicrographs were made from the best ones.

Results

Microsporogenesis and male gametophyte

Results showed that the anther of *Senecio glaucus* is tetrasporangiate (Fig. 1). Primary sporogenous cells developed directly as microsporocytes (Figs. 2,3). Meiosis, in each microsporocyte, results in a microspore tetrad via prophase I (Fig. 4), metaphase I (Fig. 5), anaphase I (Fig. 6), telophase I (Fig. 7), prophase II, metaphase II (Figs. 8, 9), anaphase II, and telophase II (Fig. 10). No cell wall is detected between the two newly formed nuclei at telophase I (Fig. 7). The cytokinesis is of the simultaneous type. The tetrads are mostly tetrahedral (Fig. 11) and rarely isobilateral (Fig. 12). Sometimes callose is well discerned around the tetrad and between each monad that is visible as clear layer without staining in used method (Fig. 11). Microspores in the two neighboring sporangia are synchronized in development. The microspore released from tetrad is not vacuolated, it has a dense cytoplasm and is somewhat irregular in shape, with a prominent and centrally placed nucleus (Fig. 13). As the central vacuole develops, the nucleus takes up a peripheral position (Fig. 14), i.e., a large vacuole squashes the cytoplasm together with the nucleus toward the microspore margin.

At the microspore stage, in each sporangium, several microspores increase their size and vacuolate dramatically. The other microspores are smaller and have condensed cytoplasm (Figs. 14-17). In the smaller microspores, nucleus then divided by mitosis into two unequal nuclei, a large vegetative and small generative one and gave rise to a bi-nucleate pollen grain (Fig. 17), further a two-cell one. In the stage of mature pollen grain the larger and vacuolated pollen grains were degenerated, so that few of them are visible. After staining of released pollen grains, only normal pollen grains are visible (Fig. 18). It seems that many microspores were produced in each sporangium but most of them are infertile, with thin layer of exine that are degenerated during later developmental stages. It seems that many microspores were produced in each sporangium but the most of them are infertile with weakly produced exine. They were degenerated during the late developmental stages. It means that there is nutritional competition that tends to apoptosis and cause to decrease pollen number but ensure their nutrition and development.

Formation of anther wall

At an early stage of development, five to eight rows of archesporial cells differentiate beneath the epidermis of the anthers. The archesporial cells are recognizable by their dense cytoplasm and conspicuous nuclei. These cells are divided periclinally, which leads to formation of outer primary parietal cells and inner primary sporogenous cells. The anther wall derived from a parietal cell layer consists of four layers; from the exterior: the epidermis, endothecium, middle layer and tapetum layer (Figs. 2-4). The tapetal cells are uni-nucleate or bi-nucleate at the stage of microsporocyte and they show radial elongation and intrude into the anther locule (Fig. 2). They undergo rapid mitosis and achieve a high level of polyploidy (Fig. 3), indicating high metabolic activity, which is analogous to the behavior of antipodal cells in the embryo sac. When microspores are released from tetrads, extensions of tapetal cells enter the anther locule (Figs. 13-18). At the stage of unicellular or bicellular pollen grains,

the tapetal cells degenerate, and only relics of the tapetum are left (Figs. 16, 17). Tapetal driven parts are visible at the stage of unicellular pollen grains (Fig. 16). At the early stages of pollen development the tapetum belongs to secretory type apparently, but in the late stages of development becomes more or less to amoeboid type. The middle layer is developed at the early stages of microsporogenesis (Figs. 2-4) but it is ephemeral and degenerated at the late stages (Figs. 11, 12). At the stage of microspores, in the endothecium fibrous thickenings have not developed yet and its cells are still alive (Figs. 16, 17). However, at the late stage of bicellular pollen grains, their development was completed.

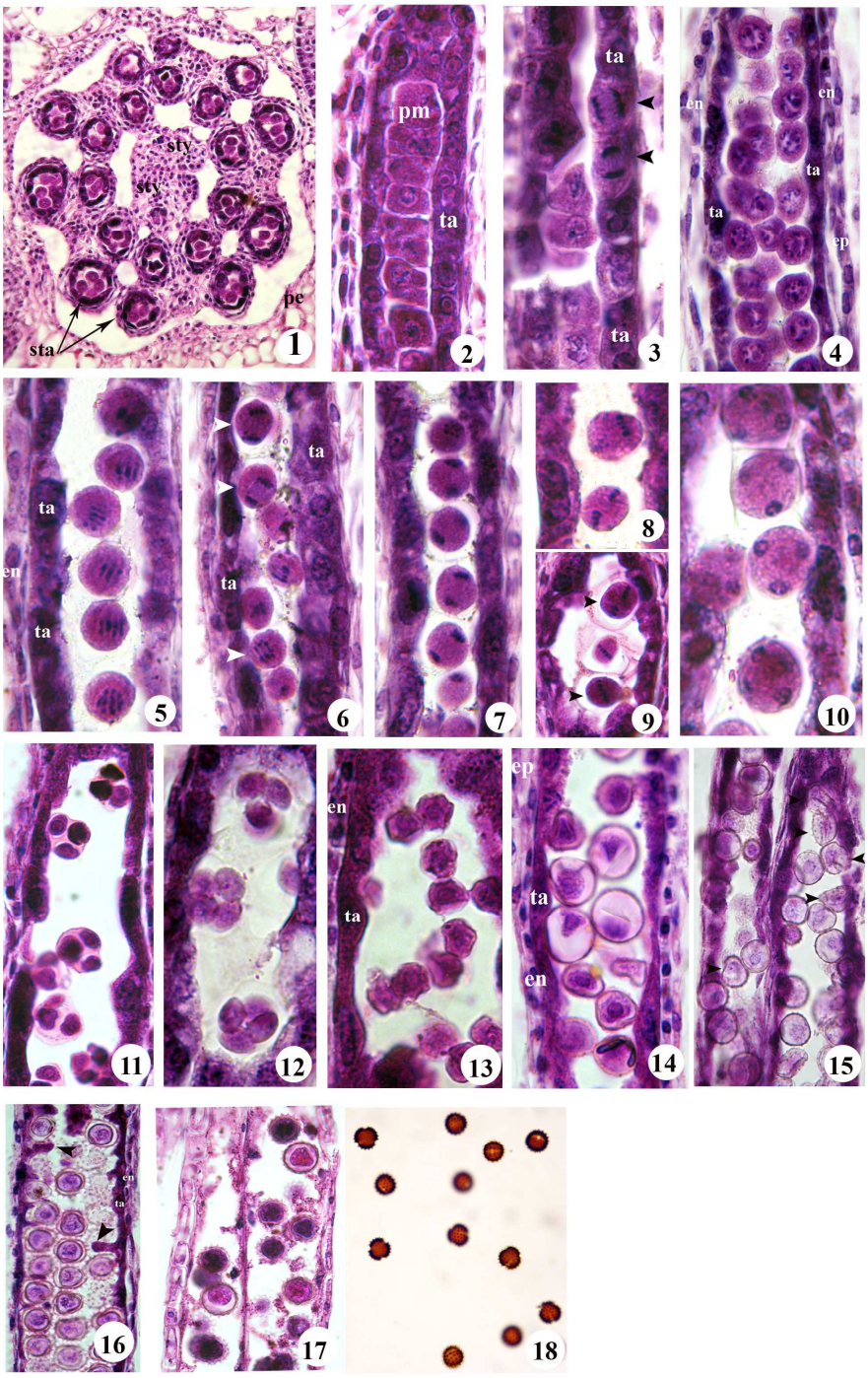
Megasporogenesis and female gametophyte development

The ovary is monocarpelate and unilocular with a basal placenta and an ovule (Figs. 19, 20). The type of ovule is anatropous and unitegmic (Fig. 20). During early ovular development, a single archesporial cell is present hypodermaly (Fig. 19). It enlarges and differentiates from the neighboring cells in the nucellus and then become the megaspore mother cell (mmc) (Fig. 20). Therefore the ovule is tenuinucellate (Fig. 20). Megaspore mother cell grows in size and enters meiosis, produces dyad (Figs. 21, 22) and finally results in a linear (Figs. 24, 25) and T-shaped tetrad of megaspores, the chalazal one of which is functional. The functional megaspore undergoes three successive mitotic divisions, and in result eight-nucleate embryo sac was produced (Fig. 26). Cell formation takes place in the embryo sac resulting in a mature embryo sac (Fig. 27). Cell formation of the embryo sac occurs during the migration of the polar nuclei and follows the *Polygonum* type.

In the mature embryo sac, the egg cell can be ambiguously distinguished from the two synergids by its relative position (Fig. 27). Two free polar nuclei were located at the center of embryo sac that came toward the egg apparatus, fussing took place near the egg apparatus and just prior to fertilization and secondary nucleus was resulted. Before the differentiation of the micropylar cells into egg cell and two synergids, the two polar nuclei had already fused to form a large secondary nucleus (Fig. 28). The polar nuclei are visible in the center of embryo sac that are fussing just before fertilization and produced a secondary nucleus with a large vacuole, and many cytoplasmic organelles. The antipodal cells increased in number and did not degenerate in the studied flowers (Fig. 29). Secondary nucleus migrated toward the egg apparatus (Fig. 30). In the all studied florets, antipodal cells continued cell division after embryo sac maturation (Figs. 29, 30). Legitimate embryo is formed from the zygote and embryogenesis is following to Asterad type.

Figs. 1-18 (on the right)**.** Microsporogenesis, male gametogenesis and development of anther wall in *Senecio glaucus*. **(1)** Trans section of young floret with five tetrasporangiate stamen. **(2)** Longitudinal section of anther showing anther wall, tapetum layer (ta) and pollen mother cells (pm). **(3)** Longitudinal section of anther showing mitosis in tapetum layer cells and meiosis in pollen mother cells. **(4-12)** Showing various stages of meiosis in microsporocytes. **(4)** Prophase I. **(5)** Metaphase I. **(6)** Anaphase I. **(7)** Telophase I. **(8, 9)** Metaphase II. **(10)** Telophase II. **(11)** Tetrahedral microspore tetrads. **(12)** Tetragonal microspore tetrads. **(13)** Microspores just released from tetrads. **(14)** Some microspores vacuolated considerably. **(15)** Larger and more vacuolated microspores are degenerating (arrows). **(16)** Remaining pollen grains and periplasmodial extension of tapetum (arrow). **(17)** Formation of pollen coats and maturation of pollen grains. **(18)** Mature tricolpate pollen grains. Scale bars = 20 µm.

ep - epidermis; **en** - endothecium; **sta** - stamen; **ta** - tapetum layer; **pm** - pollen mother cells.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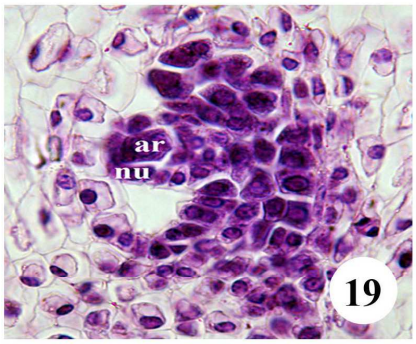
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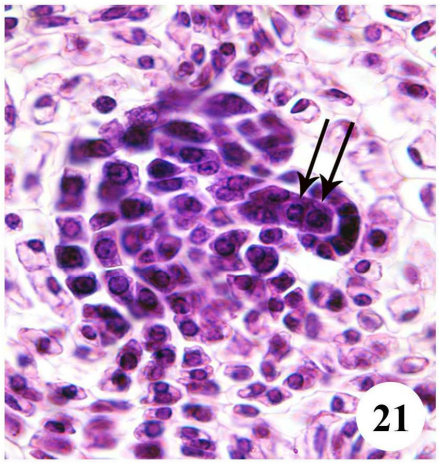
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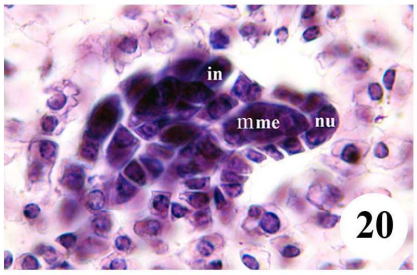
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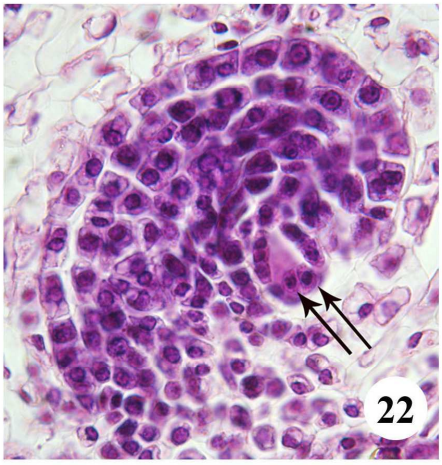
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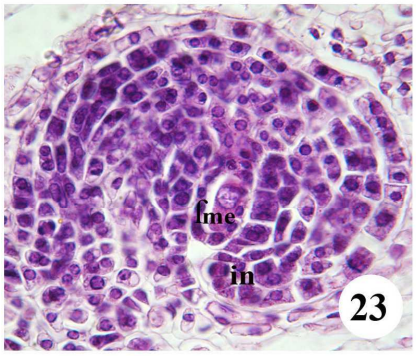
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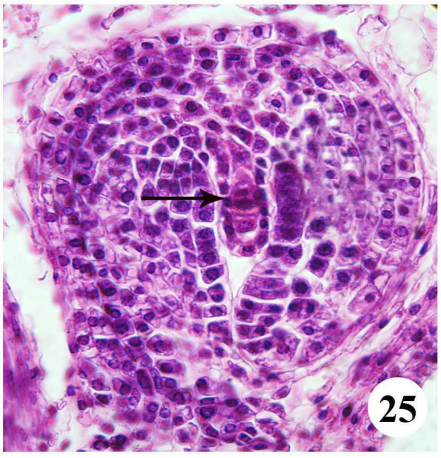
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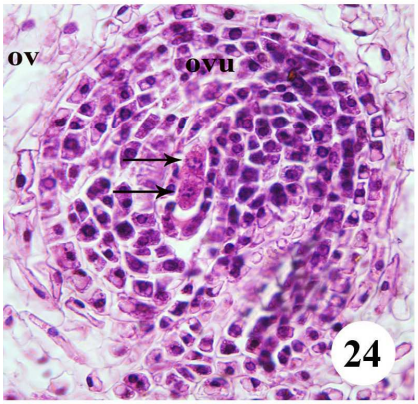
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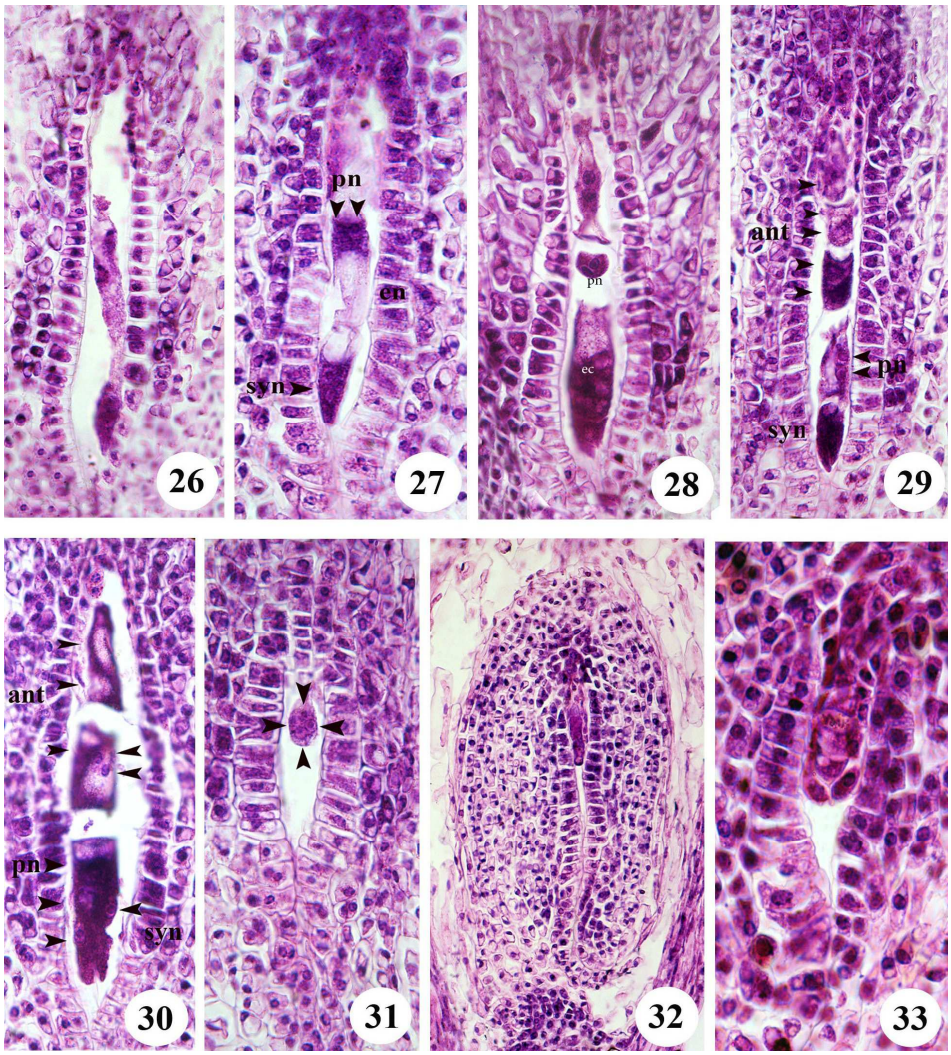

Figs. 19-33. Megasporogenesis and megagametophyte development in *Senecio glaucus*. **(19)** Young ovule with archesporial cells and nucellus. **(20)** Ovule with megaspore mother cell. **(21)** Megaspore mother cell during mitosis. **(22)** T-shaped megaspore tetrad (arrows). **(23)** Megaspore mother cell (mmc). **(24)** First meiosis resulted in formation of dyad cells (arrows). **(25)** Linear megaspore tetrad. **(26)** Eight-nucleate embryo sac. **(27)** Embryo sac at the stage of cellulization. **(28)** Maturing embryo sac that showed deveoping egg apparatus (eg), fused polar nuclei and antipodal cells. **(29)** Maturing embryo sac that showed increasing number of antipodal cells up to eight (arrows, ant). **(30)** Mature embryo sac showing two synergids (sy), an Egg Cell (ec), two polar nuclei (arrows, PN) that are migrating toward the egg apparatus. Embryo sac showed fusion of polar nuclei (pn) attached to egg apparatus. **(31, 32)** A mature ovule with an antipodal embryo was formed at the chalazal pole. **(33)** Antipodal embryo located at the chalazal end, opposite the micropylar end.

ar - archeosporial cell; **nu** - nucellus; **mc** - megaspore mother cell; **in** - integument; **fme** - functional chalazal megaspore; **ov** - ovary; **ovu** - ovule; **pn** - polar nuclei; **ant** - antipodal cells; **syn** - synergids. Scale bars = 50µm.

Based on our observation and results, at least in the 30% of florets antipodal cells cause to form a globular embryo and an attached suspensor (Figs. 31-33). In this case egg apparatus was degenerated and are not visible in the micropylar end of embryo sac (Fig. 32). Consequently the embryo was elongated and resulting to form a long and relatively irregular embryo (Fig. 32). Growth and development of antipodal embryo tend to form a more developed embryo in the chalazal end of embryo sac (Fig. 33). It seems that this should be considered as a type of Apomixis due to antipodal cell activation.

Discussion

Anther and male gametophyte

In the studied species the development of the four-layered anther wall follows the dicotelydonous-type (DAVIS 1966; WATSON & DALLWITZ 1992) but in late stages, after the stage of microspore tetrads formation, it consists of three layers because the middle layer is ephemeral and degenerates during the meiosis in microspore mother cells (mmcs), and it already does not exist at the formation of microspore tetrads. The presence of middle layer was reported by RANGASWAMY and PULLAIAH (1986) but it seems that middle layer was degenerated at late stage of anther development, in the stages of pollen mother cell meiosis. The archesporial cells are recognizable by their dense cytoplasm and conspicuous nuclei. These cells are divided periclinaly, forming outer primary parietal cells and inner primary sporogenous cells, that is accordance with a prior report (XUE and LI 2005). The endothelial fibrous thickening is not as clearly expressed as they are observed in most representatives of Asteraceae (YURUKOVA-GRANCHAROVA et al. 2006; CHAUDHURY et al. 2009). A sharp correlation was observed between the meiotic division in Pollen Mother Cells (PMCs) and the development of anther's tapetum that was reported for other Asteraceae members (GUSTAFSSON 1946).

The tapetum cells achieved a high level of polyploidy, indicating high metabolic activity, which is analogous to the behavior of antipodals in the embryo sac (MAHESHWARI 1950). In the Angiosperms two basic types of the anther tapetum are recognized (PACINI et al. 1985): secretory (parietal) and amoeboid (periplasmodial, invasive). In *Senecio glaucus* the tapetum layer showed multiplication of the nuclei in its cells (2-3 nuclei) and it differentiates in to amoeboid (plasmodial) ones so that its periplasmodial extensions are produced toward the center of anther locule (Fig. 16). Amoeboid tapetum was also reported for *Senecio tenuifolius* (LAKSHMI & PULLAIAH 1979) and also for other representatives of Asteraceae family (DAVIS 1966).

In *Senecio glaucus*, the primary sporogenous cells directly acted as pollen mother cells (PMCs), as indicated by the single row of PMCs in the anther locule (Fig. 2). Few plant species exhibit such feature (HU 1982). Unfortunately the significance of this type of PMC development in plant phylogeny is still unknown (PAN et al. 1997).

Meiosis in each microsporocyte results in a microspore tetrad. The tetrads are mostly both tetrahedral and isobilateral. Tetrahedral and decussate tetrads were also reported in *Senecio tenuifolius* (LAKSHMI & PULLAIAH, 1979). Microspores in the two neighboring sporangia are synchronizing in development. The microspores are irregular in shape, with a prominent and centrally placed nucleus. The nucleus is then divided by the mitosis and gives rise to a two-nucleate pollen grain, further two-cell one that is different from the results of Lakshmi & PULLAIAH (1979) that reported that pollen grains are 3-celled when shed.

Ovule and female gametophyte

In this species, the chalazal megaspore of the tetrahedral or T-shaped tetrad gives rise to *Polygonum* (monosporic) type of embryo sac as described for more than 70% of angiosperm (MAHESHWARI 1950; BATYGINA 1987). The other three megaspores were degenerated rapidly. Linear tetrads were reported by prior researches (RANGASWAMY & PULLAIAH 1986, 1979; KAPIL & BHATNAGAR 1981). Remaining megaspore produced 8-nucleate embryo sac and then mature embryo sac. In mature embryo sac three cells were differentiated at the micropylar end that consists of an Oosphere and two Synergids. In this study, both synergids were observed in the mature embryo sac prior to pollination and fertilization, possibly due to the fact that *Senecio glaucus* is an allogamous species. In an autogamous plant, the pollination and fertilization that gives rise to degeneration of one of the synergids is probably finished before the opening of the flower, and then one of the synergids should disappear few days after the opening of the flower (SOUZA et al. 2002)..

In Polygonum type of embryo sac, the antipodal cells are located on the opposite side of the egg cell, usually three, and frequently varying in size (MAHESHWARI 1950; CAMERON & PRAKASH 1994; XIAO & YUAN 2006) and in number, as recorded in the Asteraceae family (RANGASWAMY & PULLAIAH 1986). Our results indicate that in *S. glaucus* two types of florets and also two kind of embryogenesis are in current. In some florets the number of antipodal cells were increased to eight. In these florets, no specific function during reproduction has been attributed to the antipodals, but they are thought to be involved in the import of nutrients to the embryo sac (DIBOLL 1968). In this case, in the mature embryo sac of *S. glaucus*, eight antipodal cells were visible and embryogenesis is resulting from zygote development after fertilization.

In about 30% of samples, antipodal cells were divided and their cell numbers were increased considerably. Antipodal cells cause to form a suspensor and globular embryo that were developed later. In contrast, egg apparatus were degenerated, probably due to underutilization of egg cell. This should be considered as exceptional embryogenesis. Although apomixis was reported for several species of Asteraceae (DAVIS 1968; PULLAIAH 1979), but this is the first report about antipodal embryogenesis in *S. glaucus* that is also rare in the plants (SOLNTSEVA 1999). Within the family Asreraceae, antipodal apogamy was

established in bulgarian populations of *Achillea setacea* (TERZIJSKI et al. 1996; YURUKOVA-GRANCHAROVA et al. 2002).

The present embryological study reveals the peculiarities of the male and female generative organs of *Senecio glaucus* According our bibliographical studies, this is the first report in this aspect in this species and the results received enrich the embryological characteristic of the genus *Senecio*.

The mentioned peculiarities of the male and female generative sphere established during the study reveal a specialization of *S. glaucus*, in particular regarding the female gametophyte expressed in the following features: anatropous, tenuinucellate, unitegmic ovule; one-celled female archesporium,.

Legitimate embryo is formed from the zygote and embryogenesis is accordance to Asterad type with high frequency of apomixis that was reported in the different genera of Asteraceae family (CLAUSEN 1954). The presence of apomixis (gametophytic apomixis - formation of antipodal embryo) in the studied species established during the study show a high plasticity of the female generative sphere. On the other hand, the ratio of apomictic embryos (30 % of the studied florets) permits to consider *S. glaucus* a facultatively apomictic species in which the sexual reproduction predominates.

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