FEMALE GAMETOPHYTE, EMBRYO- AND ENDOSPERMOGENESIS IN ALCHEMILLA GLABRA (ROSACEAE: ROSOIDEAE)

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Abstract

A cytoembryological study on native Bulgarian populations of *Alchemilla glabra* was carried out for the first time. The peculiarities of the female gametophyte development, embryo- and endospermogenesis were investigated. The legitimate embryo sac develops after Polygonum (monosporic)-type. In single ovules an apospory according to Hieracium-type was also registered. A tendency to forming diplosporous embryo sacs was observed. The embryogenesis proceeds according to Asterad-type. This study reveals *Alchemilla glabra* as a facultative apomictic species. An assumption regarding relationships between the characteristics of the reproduction patterns and the wide spreading of this species is suggested.

Key words: *Alchemilla*, apospory, female gametophyte, embryology, embryogenesis, endospermogenesis

Introduction. *Alchemilla* L. is one of the most difficult taxonomically genus in the family Rosaceae, subfamily Rosoideae. The genus consists of 433 wild species in the European flora as the number of newly described taxa continuously increases [7]. In Bulgarian flora the genus *Alchemilla* is represented with 35 species [2] belonging to the section *Alchemilla*, namely subsections *Calycanthum* Rothm., *Chiropodium* Rothm., *Heliodrosium* Rothm. while *A. glabra* Neygenf. – to the Heliodrosium subsection, series Vulgares.

The species of the genus find wide application in haematology and gynaecology due to a high content of flavonoids and tannins [3, 4] that determine great interest as well as aggressive collecting of plants from their native habitats. Because
of their sustainable use as natural bioresources good knowledge of the peculiarities of the reproductive potential of the species Alchemilla is necessary.

The scientific information on the reproductive processes shows that the apomixis and high ploid levels \((2n = 96, C.100, 102–110)\) get together in the genus Alchemilla \([5–7]\). Nearly eighty species from this genus and six of them of subsection Heliodrosium are studied embryologically up to now and generative apospory, unreduced parthenogenesis, nuellar embryony and unreduced apogamety are announced \([6, 8]\). According to most authors \([5, 6, 9]\) the obligatory apomixis is characteristic of the genus Alchemilla. Glazunova \([10, 11]\) allows the possibility for a coexistence of the sexual and asexual reproductive programmes and clearly suggests that the genus Alchemilla should be considered as a big agamic complex.

Despite its wide spreading the species \([1]\) A. glabra is poorly studied embryologically \([7]\). It is important to notice that the literature data on the reproductive features studied embryologically of the species Alchemilla until now are illustrated only with drawings and more objective illustrative material like photos is missing.

The aim of the present work is to investigate the peculiarities of the structures and processes of the female generative sphere, the type of reproduction and the character of the apomixis in A. glabra, as part of a more wide embryological study undertaken on the Bulgarian representatives of the genus Alchemilla.

**Material and methods.** Alchemilla glabra Neygenf. from two natural Bulgarian clone populations (Rila mountain, Borovetz, near Yastrebetz lift station and Vitosha mountain, near Goli vrah lift station) were investigated embryologically. The material of the study (flower buds and opened flowers in various stages of development) was fixed in FAA mixture – formalin: glacial acetic acid: ethanol (5 : 5 : 90 parts with 70% ethanol) and treated according to classical paraffin methods.

Serial sections cut 6–14 µm thick, were stained with Heidenhein’s haematoxylin and were mounted in Canada balsam. The observations were carried out with light microscope “Amplival”. The microphotographs were made with Canon PowerShot A 610 camera.

**Results and discussion.** Into the one-loculate apocarpous gynoecium of A. glabra a single crassinucellate unitegmic ovule forms basally and reaches to maturity \([10, 12]\). In the earlier phases of the ovule ontogenesis a multicellular archesporium forms hypodermally in it and a single integument starts to differentiate. This pattern of the ovule ontogenesis is shown as typical of other genera of the family Rosaceae such as Potentilla, Rubus and Fragaria \([8]\).

The primary archesporial tissue is surrounded with one-layered nuellar epidermis that further transforms into multilayered one in its apical part because of several mitotic divisions forming by this way the so-called “nuellar cap” \([5, 12]\). In the well developed ovule the micropyle is absent due to the adhesion of the ends of integument together with the cells of the nuellar cap \([12, 13]\).

Archesporogenesis runs with separation from four to seven layers of parietal
cells that is typical of the most taxa of Rosaceae [8,14]. After several consecutive mitotic divisions the archesporium is composed as 3-layered reproductive complex [15] and consists of a middle row whose cells divide periclinally and two lateral rows of cells divide in a diagonal direction.

In most cases the chalazal cell of the middle row of the archesporial tissue functions as a megaspore mother cell (MMC). Later on, a normal meiosis is often established, leading to a formation of linear megaspore tetrad in the ovule. This process observed during the study is considered as an exception in other species of the genus *Alchemilla* [5,15]. Rarely, the chalazal cells of the lateral layers of the archesporium complex show a tendency to the meiosis too (a prophase I is registered).

In some ovules the epichalazal cell that is larger and strongly vacualized than the other cells of the megaspore tetrad functions as embryo sac mother cell (EMC). The three sister cells of the tetrad that are darkly stained degenerate rapidly.

In the chalazal part of the ovule at the stage of 2-nuclear ES a hypostasis forms that consists of cells with thick walls and strongly stained cytoplasm [8,12]. After three consecutive mitosis in the EMC an 8-nucleate embryo sac forms. Later on, a mature ES consists of 3-celled egg apparatus with a pyriform egg cell and 2 strongly lengthened synergids without filamentous apparatus, two polar nuclei in the close proximity to the egg cell and three ephemeral antipodals. It was observed that the fusion of the two polar nuclei into the central cell (secondary nucleus) of the ES is a very prolonged process. Thus ES is differentiating according to monosporic Polygonum-type so, but without converging at the polar nuclei.

Rarely, in some MMCs the meiosis is inhibited or even is missing and then they function directly as EMCs giving rise after 3 mitotic divisions to a diplosporous ES. Morphologically, the diplosporous unreduced ESs are identical with the meiotic ones [16,17].

Sporadically, in some cells of the lateral rows of the archesporium complex a peculiar somatization takes place [15] confirming the standpoint [17] that in the species with multicellular archesporium like *Alchemilla* is very difficult to put a strong boundary between the diplospory and apospory. The somatic initials after three mitosis differentiate into aposporous ESs according to the *Hieracium*-type [17,18]. In *A. glabra* the aposporous ES dissiated deeply into the chalaze co-exist together with the legitimate one in a row into the ovule. Opposed disposition, namely a parallel one announced for other species of the genus *Alchemilla* [5].

Usually, during the ES development an asynchrony in the mitotic divisions was observed as violated polarization of the elements of an egg apparatus. These features are typical of *Alchemilla* spp. [5,8], but in *A. glabra* the degeneration of embryological structures is registered in a higher degree.

Moreover, in about fifty per cent of the ovules some deviations during the archesporogenesis were observed even to a complete degeneration of the archespo-
rial cell complex. Later on, at the stages of 2-, 4-, and 8-nucleate ES degeneration processes were also established (Fig. 1).

The most stable reproductive structure in *A. glabra* is the egg cell. That is one more sign, which distinguished *A. glabra* from other investigated species of the genus [15]. The egg cell preserves its vitality as long as the other parts of the ES in degenerations – synergids, polar nuclei and antipodials.

Despite the high amount of sterile pollen [unpublished data] the penetration of the pollen tube through micropyle and fertilization of the egg cell in some ovules were registered (Fig. 2).

The first division of the zygote is transversal and further the embryo develops according to Asterad-type that is typical of the embryogenesis of the members of *Rosaceae* family or to second megarchetype after Soueges [13]. The globular embryo possesses 2-, 3-cellulate suspensor (Fig. 3).

MANDRIK [7] shows adventitious nucellar embryony in *A. alpestris* but later on this taxon was included in *A. glabra* due to a nomenclatural deviations. It must be noticed that in this study on *A. glabra* a polyembryony or adventitious embryony was not observed.

The endospermogenesis begins before the first division of the zygote. Initially, the endosperm is nuclear and preserves in this state very long time. During the latest stages of the embryogenesis the nuclear endosperm transforms into cellular one. A strong morphological diversity was observed between the free endospermal nuclei mostly in respect to the number of nucleoli in them (Fig. 3). This feature occurs when the apomictic process takes place in the Angiosperms taxa [19]. Often, the embryo degenerates before the formation of cellular endosperm.

In the globular embryo stage an endospermal haustorium’s [5] was observed (Fig. 4). For some taxa of genus *Alchemilla* belonging to the section *Heliodrosium*, as: *A. acutiloba, A. flabellata* and mainly in *A. monticola* IZMAILOW [9] announces that in the flowers the embryogenesis begins before the endospermogenesis and emphasizes that in unopened flower buds a precocious embryogenesis runs, too. In this embryological study on *A. glabra* any kind of similar tendencies was not observed.

Although the preserved possibilities for amphimictic seed production a degeneration of the embryo at different stages of its development was often observed. Some disturbances during embryogenesis are observed in a number of ovules, as deviations from the normal mitotic divisions after Asterad-type embryogenesis and a cleavage of the embryos. This is a consequence from the unbalanced embryo- and endospermogenesis that is probably connected with the polyploid character of *A. glabra* [1,20].

**Conclusion.** The results of the present study carried out on *Alchemilla glabra* show extreme imbalances of the processes and the instability in the structures of the female reproductive sphere of the species. During the archesporogenesis a number of different deviations and even inhibition or missing of meiosis
Fig. 1. Mature ES in degeneration

Fig. 2. Zygote with free endospermal nuclei

Fig. 3. Embryo at globular stage

Fig. 4. Endospermal nuclei with more than one nucleolus; endospermal haustoria
were established. These disturbances and the deviations in ES forming and embryogenesis, together with polyploidy are significant markers for a tendency to apomictic reproduction.

The registered cases of fertilization show saved great potential of amphimictic seed reproduction (known in apomictic species as residual sexuality). The data received show that Bulgarian representatives of *A. glabra* may be characterized as amphy-apomictic species that combines sexual reproduction with aposporous apomixis. Despite of its good opportunities for vegetative propagation, this mode of reproduction is a possible reason for wide spreading of this species in Bulgarian flora.

REFERENCES


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