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Development of Female Gametophyte in *Gagea villosa*

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Summary This is the first study in which gynoeceum, megasporogenesis, megagametogenesis and female gametophyte of *Gagea villosa* were examined cytologically and histologically by using light microscopy techniques. Ovules of *G. villosa* are of anatropous, bitegmic and tenuinucellate type. Inner integument forms the micropyle. Embryo sac development is of bisporic Endymion type. Polar nuclei fuse before fertilization to form a secondary nucleus near the antipodals.

Key words *Gagea villosa*, Liliaceae, Megasporogenesis, Megagametogenesis.

The Liliaceae family is represented by approximately 250 genera and 3,500 species in the world. 36 genera and 461 species of them are found in Turkey. It is a cosmopolitan family. It shows more natural distribution in tropical and temperate regions. This family includes both medicinal and important ornamental plants. There are 26 species of the genus *Gagea* in Turkey (Zarrei *et al.* 2007). *Gagea villosa* is distributed through Turkey, Europe, North Africa, the Crimea, the Caucasus, Iran, and Palestine.

The genus *Gagea* has been the object of karyological (Başak 1990, Özhatay 2002, Peruzzi 2008), systematical (Davis 1966, Rechinger 1986, Başak 1990, Ali 2006, Levichev 2006, Peruzzi 2006, Zarrei *et al.* 2007, Eker *et al.* 2008), morphological (Başak 1990, Kosenko 1999, Karaca *et al.* 2007, Schnittler *et al.* 2009), phylogenetical (Peterson *et al.* 2004, Fay *et al.* 2006, Peruzzi *et al.* 2008), and molecular (Zhang *et al.* 1995, Buzek *et al.* 1998, Leitch *et al.* 2007) studies.

Embryological studies done with genus *Gagea* are rather limited. Studies about the development of the embryo sac in *Gagea lutea* (Nemec 1912, Stenar 1927, Greilhuber *et al.* 2000), *G. fascicularis* (Stenar 1927), *G. bohemica*, *G. granatellii*, *G. chrysantha* (Caparelli *et al.* 2006), *G. bohemica* (Vardar *et al.* 2012), *G. chlorantha*, *G. tenuifolia* (Gvaladze 1974), *G. chomutovae*, *G. olgae*, *G. parva* (Romanov 1961), *G. fascicularis* (Joshi 1946), *G. graminifolia*, *G. tenera* (Romanov 1936), *G. kashmirensis* (Koul *et al.* 1969), *G. minima*, *G. spathacea* (Westergaard 1936), *G. ova* (Romanov 1936), *G. persica* (Saddiqi and Hashmi 1975), *G. reticulate* (Koul and Wakhlu 1985), and *G. stipitata* (Koul *et al.* 1976) were reported.

Gagea villosa was studied karyologically. The chromosome number was determined as $2n=24$, 36 and 48 in samples (Başak 1990, Özhatay 2002, Peruzzi 2008). The pollen morphology of *Gagea villosa* was examined using light and scanning electron microscopy by Karaca *et al.* (2007). The pollen grains were heteropolar and oblate. Apertures are sulcate and ornamentation is reticulate (Karaca *et al.* 2007). Other studies done on *G. villosa* were phylogenetical (Peterson *et al.* 2004), systematical, and morphological (Zarrei *et al.* 2007) studies. Cytological and embryological features of *G. villosa* have not been studied yet.

In recent years, populations of this species in the Edirne Region are rapidly eradicated under the name of meadow improvement. According to Başak (1990), this species was distributed mostly

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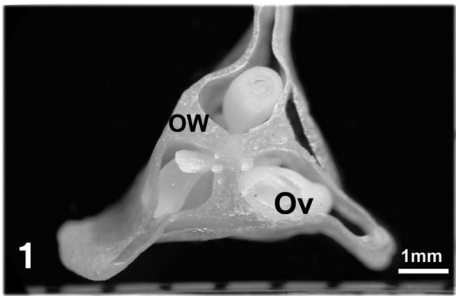

Fig. 1. Cross section of *Gagea villosa*'s mature ovary (OW, ovary wall; Ov, ovule).

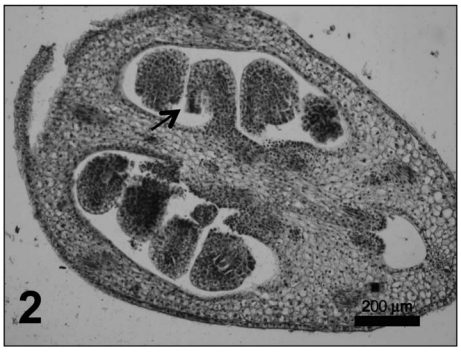

Fig. 2. Longitudinal section of *Gagea villosa*'s mature ovary; anatropous ovules (arrow).

under *Paliurus* sp. (Thorn) in meadows of villages or roadsides in Edirne. Thorns are rapidly eradicated to improve meadows in villages. The habitat of *G. villosa* is disappearing rapidly as a result of these studies.

The aim of this study is to determine the development of megasporangium, megasporogenesis, megagametogenesis and organization of the mature embryo sac of *G. villosa*. This study is also an attempt toward a better understanding of taxonomic relationships with closely related taxa within the Liliaceae and to contribute to efforts to protect this species *in vitro*.

Materials and methods

In this study, *G. villosa* plants (Fig. 1a) were collected from Havsa village of Edirne A1 (E) in European Turkey, between March and May of 2010–2012. They were brought to the Botanical Garden of Trakya University. Voucher specimens were placed in the Herbarium of Trakya University (EDTU). Ovaries were examined under an Olympus SZ61 stereomicroscope. For cyto-histological studies, flowers and buds were fixed in Carnoy's fluid (3 : 1, ethyl alcohol : acetic acid). Customary methods of dehydration, infiltration, paraffin embedding, microtoming and staining were followed (Johansen 1940). Serial sections of ovaries were cut at a thickness of 6–15 μm with a Leica RM2125 RT mikrotom and stained with Delafield's hematoxylin (Jensen 1962). Slides were examined with an Olympus CX31 microscope and photographed by an Olympus E330 camera.

Results

Gynoecium

Gynoeciums of *Gagea villosa*, which were between 3–16 mm in length, were dissected from

buds and flowers. *G. villosa* has the trilocular, syncarpous, superior ovary. It is observed that 15–30 ovules are marginal-central placented (Fig. 1).

Development of megasporangium

The ovules are bitegmic. Integument protuberances were seen in the ovules at preceding phases when the megaspore mother cell was at the beginning of prophase I, integument protuberances were begun to develop in the ovules (Fig. 3a, b). During the megasporogenesis integuments develop (Fig. 3c) and the ovule becomes anatropous (Fig. 3d). Micropyle is formed by inner integument. The inner and outer integuments are two- to three-layered around the micropyle (Fig. 3c).

Megasporogenesis

Ovules of *G. villosa* are teninucellate (Fig. 3a, b). Regular meiosis I is seen in MMC. Zygonema, bouquet stage (Fig. 4a), diakinesis, (Fig. 4b, c), metaphase I (Fig. 4d), anaphase I (Fig. 5a) are regular. Karyokinesis is followed by cytokinesis in telophase I. It divides meiotically forming one dyad (Fig. 5b). Later, the chalazal megaspore degenerates and the micropylar megaspore functions (Fig. 5c, d), forming the active megaspore.

Megagametogenesis and female gametophyte

The embryo sac of *G. villosa* is of the *Endymion* type. It has eight nuclei. Three mitotic divisions of the micropylar megaspore lead to the formation of two (Fig. 6a), four (Fig. 6c) and eight nucleate embryo sacs (Fig. 6d). Also, metaphase can be seen before the four-nucleate embryo sac stage (Fig. 6b). After the third division, there are eight nuclei in the embryo sac. A large distance between the two groups of eight nuclei was observed in the eight-nucleate embryo sac (Fig. 6d). After this stage, organization of the nuclei began in the embryo sac: three at the chalazal end (antipodals); three at the micropylar end (egg apparatus) and two central polar nuclei (Fig. 7a–c). Then, polar nuclei migrate toward the antipodals (Fig. 7d) and fuse before fertilization (Fig. 8a) to form secondary nuclei near the antipodals (Fig. 8b, c).

Mature embryo sac

Three antipodal cells in the chalazal side remain preserved in the mature female gametophyte (Fig. 8a). Two polar nuclei fuse before fertilization and form a secondary nucleus near the antipodal cells (Fig. 8b, c). The egg apparatus of the mature embryo sac has one egg cell (Fig. 8d) and two synergid cells (Fig. 9a, b). The inner integument cells of the mature embryo sac at the micropylar side are formed by three to five cell layers (Fig. 9a). The egg cell nucleus is observed in the chalazal side (Fig. 9c). Synergid cells have a dense cytoplasm in the micropylar side. Large vacuoles were observed on the chalazal side and nuclei were seen on the micropylar poles of the synergids (Fig. 9d).

Discussion

In this study, the developmental stages of female gametophyte in *G. villosa* grown naturally in Edirne-Havsa village are presented using light microscopy techniques. *G. villosa* is represented by *G. villosa* var. *villosa* in Turkey (Başak 1990).

G. villosa has the trilocular, syncarpous ovary, and there are numerous ovules in each carpel like the other members of the family Liliaceae (Davis 1966).

Ovules of *G. villosa* are anatropous, bitegmic and teninucellate like in other *Gagea* species studied, *e.g. G. stipitata* (Koul *et al.* 1976), *G. chrysantha*, *G. granatelli* (Caparelli *et al.* 2006), *G. bohemica* (Caparelli *et al.* 2006, Vardar *et al.* 2012). The integumetary initials appear at the

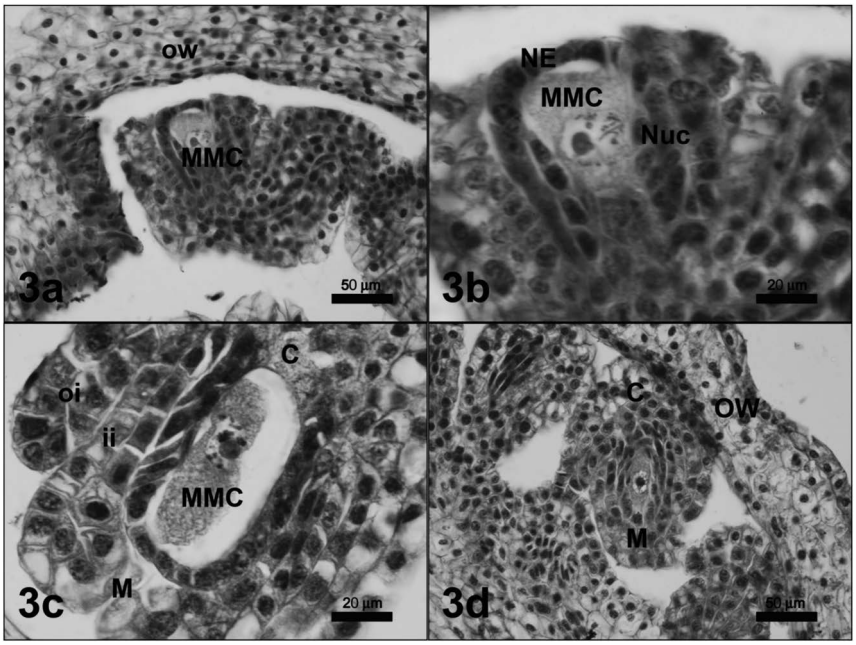

Fig. 3. a. General view of *G. villosa*'s megaspore mother cell. b. Tenuinucellate ovule of *G. villosa*. c. Prophase I. d. Phase where ovule started to turn. (C, chalaza; ii, inner integument; M, micropyle; MMC, megaspore mother cell; Nuc, nucellus; NE, nucellar epidermis; oi, outer integument; OW, ovary wall).

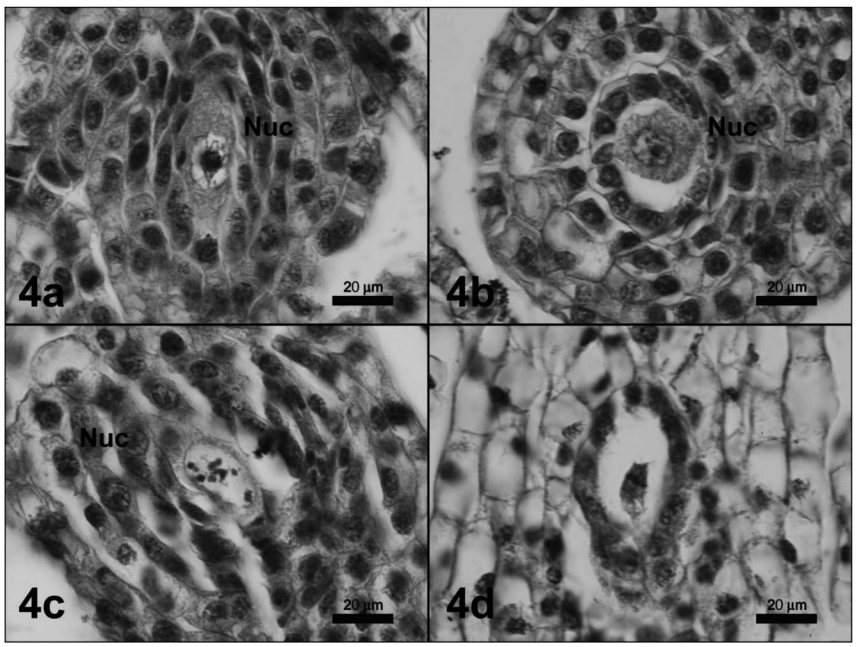

Fig. 4. a. Prophase I—Zygonema, bouquet stage. b. Diakinesis (view from above) c. Diakinesis (side view). d. Metaphase I. (Nuc, Nucellus).

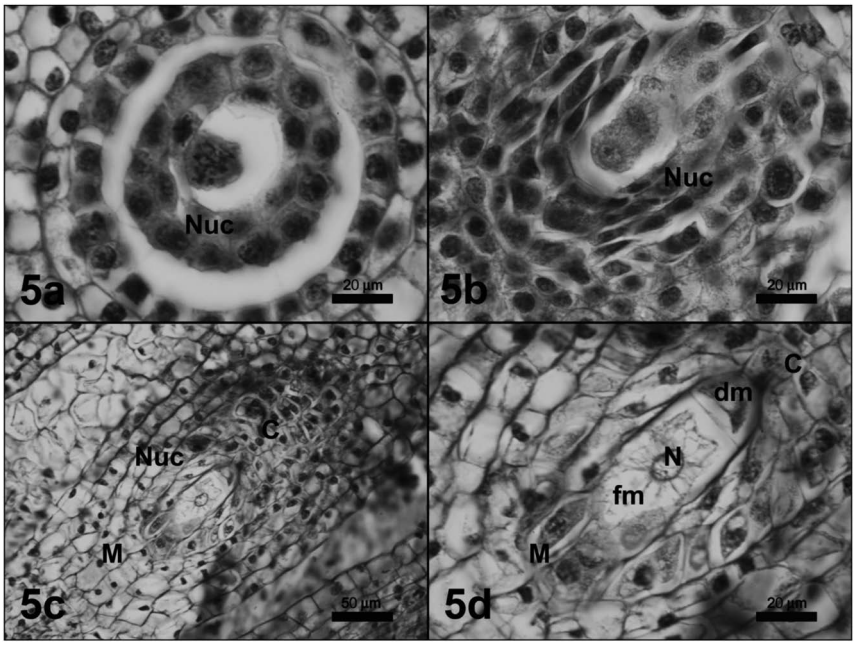

Fig. 5. a. Anaphase I. b. Diad cells in megasporogenesis. c. General view of functional and degenerated megaspores at diad phase. d. Functional and degenerated megaspores (C, chalaza; dm, degenerated microspore; fm, functional megaspore; M, micropyle; N, nucleus; Nuc, nucellus).

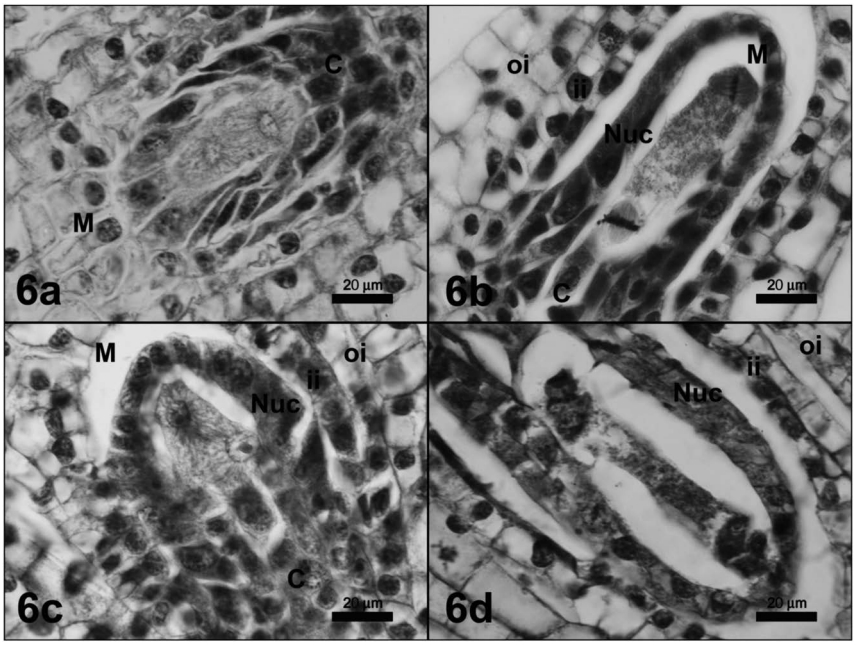

Fig. 6. Megagametogenesis a. Two-nucleate embryo sac. b. Metaphase before four-nucleate embryo sac. c. Four-nucleate embryo sac. d. Eight-nucleate embryo sac (C, chalaza; ii, inner integument; M, micropyle; Nuc, nucellus; oi, outer integument).

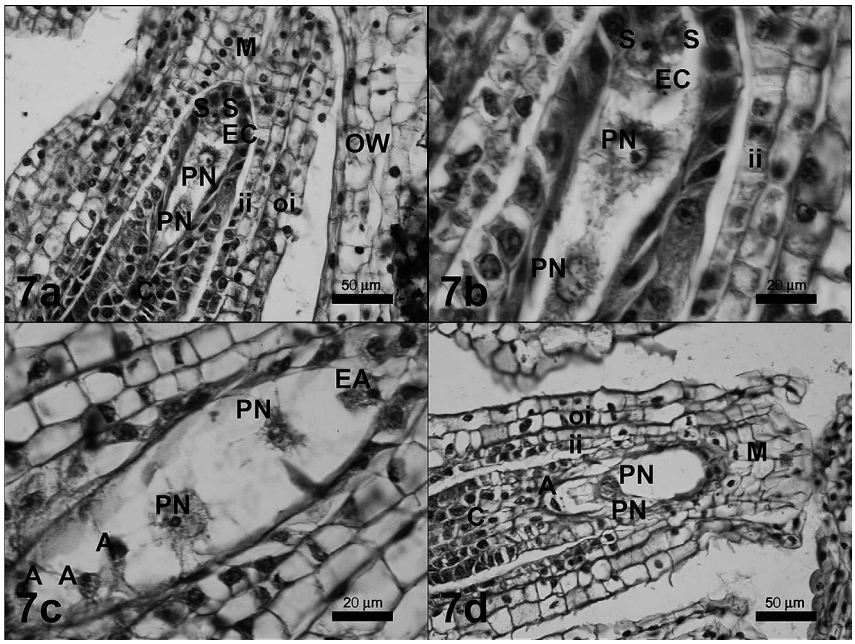

Fig. 7. a. General view of *G. villosa*'s embryo sac that started to organize. b. Polar nuclei and formation of egg apparatus in embryo sac. c. Mature embryo sac of *G. villosa*. d. General view of polar nuclei fusion in embryo sac (A, antipodal cell; C, chalaza; EA, egg apparatus; EC, egg cell; ii, inner integument; M, micropyle; oi, outer integument; OW, ovary wall; PN, polar nucleus; S, synergid cell).

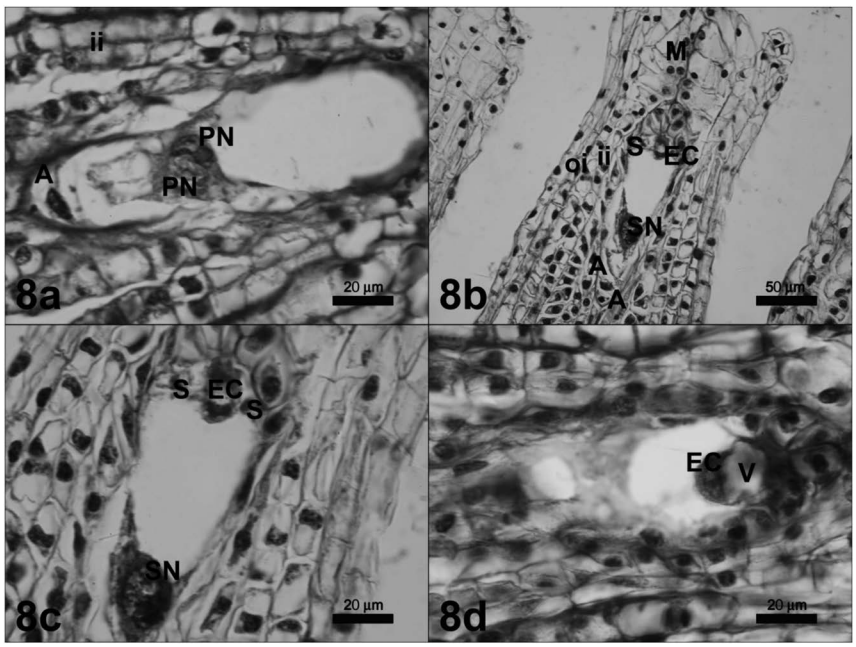

Fig. 8. a. Fusion of two polar nuclei. b. Occurence of secondary nucleus in embryo sac. c. Egg apparatus and secondary nucleus in embryo sac. d. Egg apparatus in mature embryo sac (A, antipodal cell; EC, egg cell; ii, inner integument; M, micropyle; oi, outer integument; PN, polar nucleus; S, synergid cell; SN, secondary nucleus; V, vacuole).

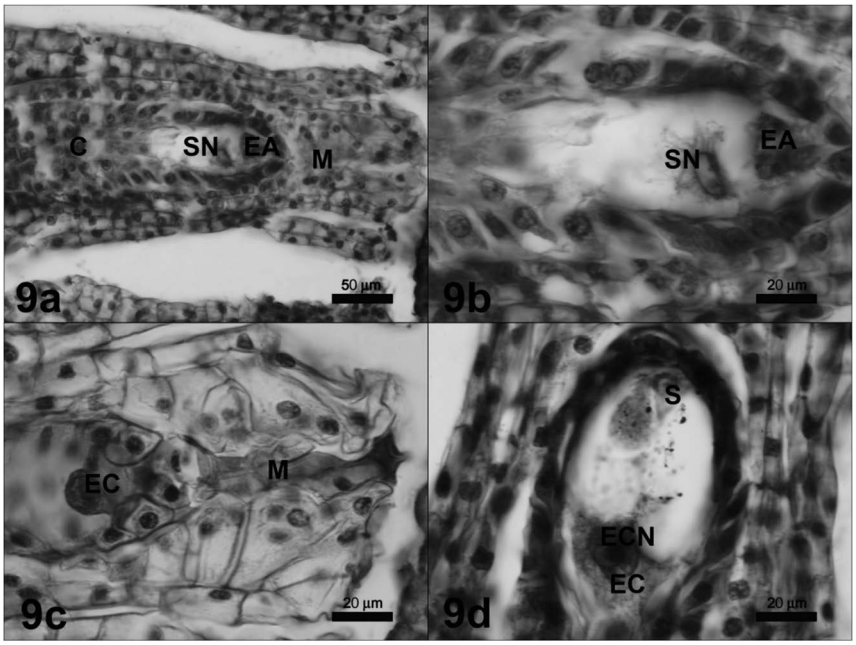

Fig. 9. a. General view of egg apparatus and secondary nucleus in mature embryo sac of *G. villosa*. b. Egg apparatus and secondary nucleus. c. Egg cell on the micropylar side of the mature embryo sac. d. Egg apparatus in mature embryo sac (C, chalaza; EA, egg apparatus; EC, egg cell; ECN, egg cell nucleus; M, micropyle; SN, secondary nucleus; S, synergid).

beginning of the megaspore mother cell stage. The micropyle is formed by the inner integument in *G. villosa* like in *G. stipitata* (Koul *et al.* 1976), *G. chrysantha*, *G. granatelli* (Caparelli *et al.* 2006) and *G. bohemica* (Caparelli *et al.* 2006, Vardar *et al.* 2012).

In *G. villosa* the archesporial cell functions directly as the mother cell like in *G. stipitata* (Koul *et al.* 1976), *G. chrysantha, G. granatelli* (Caparelli *et al.* 2006), and *G. bohemica* (Caparelli *et al.* 2006, Vardar *et al.* 2012). Cytokinesis in the megaspore mother cell of *G. villosa* follows only the first meiotic division, and the micropylar cell of the diad develops into an *Endymion* type embryo sac. In previous studies, the first meiotic division was followed by the second division of meiosis, and it led to a four-nucleate embryo sac. The four nuclei are arranged in different figures, such as in one row or on rare occasions in zigzag fashion, depending on the breadth of the embryo sac. Subsequent nuclear arrangement is 1+3. Triple fusion of nuclei was seen in *G. stipitata* (Koul *et al.* 1976), *G. chrysantha*, *G. granatelli* (Caparelli *et al.* 2006), and *G. bohemica* (Caparelli *et al.* 2006, Vardar *et al.* 2012). Afterwards, mitotic divisions in the functional megaspore result in an eight-nucleate embryo sac in most cases, such as for *G.villosa*. In some species, seven-nucleate embryo sacs were seen (Caparelli *et al.* 2006). A *Euphorbia dulcis*–type embryo sac was reported in *G. chrysantha*, *G. granatelli*, *G. Bohemica* (Caparelli *et al.* 2006), and *Frittilaria* (Koul 1976, Vardar *et al.* 2012).

A three-celled egg apparatus including one egg cell and two synergid cells is seen in the micropylar side of the embryo sac in *G. villosa*, much like in *G. stipitata* (Koul *et al.* 1976), *G. chrysantha*, *G. granatelli* (Caparelli *et al.* 2006), and *G. bohemica* (Caparelli *et al.* 2006, Vardar *et al.* 2012). Polar nuclei were situated below the antipodals and fused to form a secondary nucleus. The secondary nucleus was close to the antipodals in *G. villosa*, much like in *G. stipitata* (Koul *et al.* 1976), *G. chrysantha*, *G. granatelli* (Caparelli *et al.* 2006), and *G. bohemica* (Caparelli *et al.*

2006, Vardar *et al.* 2012). However, the nuclear content of the secondary nucleus was less in *G. villosa* compared to the others because nuclear fusions did not occur after meiosis in *G. villosa.* The number of antipodal cells was also different among the *Gagea* species. It can be two or three. This number determines whether the embryo sac is seven- or eight-nucleate. Koul *et al.* (1976) and Caparelli *et al.* (2006) reported seven- and eight-nucleated embryo sacs in the *Gagea* species. In *G. Bohemica* (Vardar *et al.* 2012), an eight-nucleated embryo sac was reported like in *G. villosa*.

The obturator, which is formed at the base of the funiculus and at the tip of the carpel margin, attracts attention during embryo sac development. Obturator formation was determined in *G. bohemica* by Vardar *et al.* (2012). This was not mentioned in other studies with the *Gagea* species by Koul *et al.* (1976) and Caparelli *et al.* (2006). In this study, the obturator was not observed in *G. villosa*.

In conclusion, the cytological and embryological characteristics of *G. villosa* were studied for the first time. The development of the female gametophyte is normal. The development and type of embryo sac are different from other studied *Gagea* species. Data gained from this study will also contribute to the embryological characteristics used in the taxonomy of Liliaceae.

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