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Microsporogenesis, pollen mitosis and pollen tube growth in *Gagea villosa* (Liliaceae)

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Abstract: In this study, *Gagea villosa* (Bieb.) Duby was investigated by using light microscopy methods in cytological and cytoembryological respects. Anthers were tetrasporangiate. Anther wall was formed with an epidermis, endothecium, middle layer and tapetum. Tapetum was glandular type and it began to degenerate when microspores released from tetrads. Tapetum cells generally have one or two nuclei. Mitosis seen in tapetum cells was generally normal but micronuclei were found in some of them. Fibrous thickenings were determined in endothecium. Microsporogenesis and pollen mitosis were generally regular. Cytokinesis was successive type. Meiosis in pollen mother cells was asynchronous in one anther locus. Mature pollen grains were 2-celled. Pollen sterility was found to be 24%. Some of the fertile pollen grains, smaller than the normal were seen at the end of the pollen mitosis. Microgametophyte development was examined *in vivo* and *in vitro*. Germination ratio of pollen grains *in vitro* was 4%. Generally swollen pollen tube tips and weak development of some curled pollen tubes were seen. Callose plug formation was seen only *in vivo* pollen tube growth.

Key words: *Gagea villosa*; Liliaceae; microsporogenesis; pollen tube

Introduction

Liliaceae family is represented by approximately 250 genera and 3500 species in the world and it is represented by 36 genera and 461 species in Turkey. It is a cosmopolitan family which shows natural distribution mostly in tropical and temperate regions. It includes both medicinal and important ornamental plants. 26 species of the genus *Gagea* are found in Turkey (Zarrei et al. 2007). *Gagea villosa* shows distribution in Turkey, Europe, North Africa, the Crimea, the Caucasus, Iran and Palestine (Başak 1990).

Embryological studies done with genus *Gagea* are rather limited. Ultrastructure and immunocytochemistry of pollen tubes in *Gagea lutea* were studied (Ignatowska & Bohdanowicz 2010). Other studies are about the development of embryo sac in *Gagea lutea* (Greilhuber et al. 2000; Nemec 2012; Stenar 1927), *G. fascicularis* (Stenar 1927), *G. bohemica, G. granatellii, G. chrysantha* (Caparelli et al. 2006), *G. bohemica* (Vardar et al. 2012), *G. chlorantha, G. tenuifolia, 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. ova* (Romanov 1936), *G. persica* (Saddiqi & Hashmi 1975), *G. reticulate* (Koul & Wakhlu 1985) and *G. stipitata* (Koul et al. 1976).

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

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 were distributed mostly under *Paliurus* sp. (a thorn bearing plant) in meadows of villages or in roadsides in Edirne. Thorned plants are rapidly eradicated to improve meadows in villages. Habitat of *G. villosa* disappears rapidly as a result of this.

The aim of this study is to determine cytological and embryological characteristics of *Gagea villosa* which grows in a limited area and in danger of extinction. It 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*.

Material and methods

In this study, anthers of *G. villosa* (Fig. 1) in various lengths were used. Materials were collected from pastures in the center of Edirne and from Havsa Village in 2010–2013. Characteristics of anthers were studied with a Olympus SZ61 stereo

Fig. 1. *Gagea villosa* (Bieb.) Duby

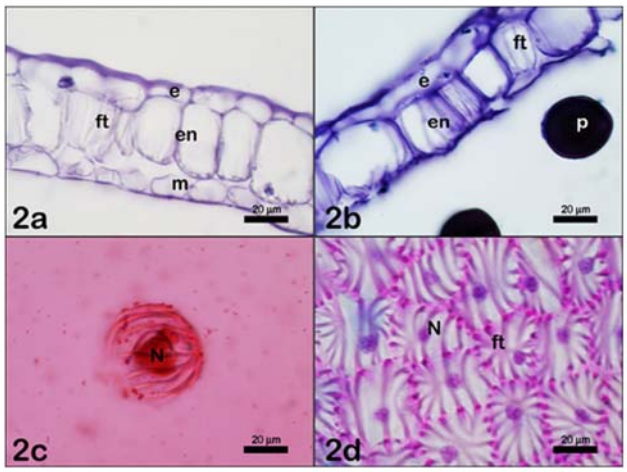

Fig. 2. Anther wall of *G. villos*a. 2a – anther wall in one-nucleated microspore phase; 2b – anther wall in mature pollen phase; 2c – young cell of endothecium layer; 2d – general view of endothecium layer and fibrous thickenings (e, epidermis; en, endothecium; ft, fibrous thickenings; m, middle layer; N, nucleus; p, pollen).

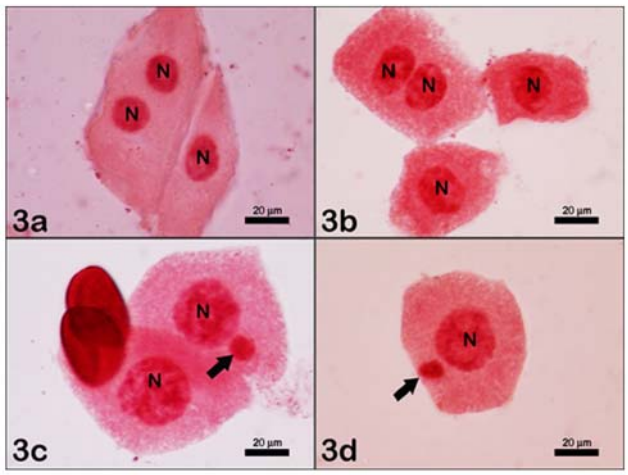

Fig. 3. Tapetum cells in *G. villosa*. 3a, b – one and two-nucleated tapetum cells; 3c, d – micronuclei in tapetum cells (arrows) (N, nucleus).

microscope. Anthers were embedded in paraffin and stained in Delafield's hematoxylene (Johansen 1940). Anther wall and phases of pollen mitosis were investigated. Different phases of microsporogenesis were investigated by using 1% aceto-orcein using the squash preperation method. Pollen viability is studied with aceto-orcein prepared in glycerine and aniline blue prepared in lactophenol (Alexander 1969; Ünal 1983). *In vitro* germination test was applied. Pollen grains from dehisced anthers were incubated in liquid germination medium (sucrose, H_3BO_4, $Ca(NO_3)_2$, $MgSO_4$ and KNO_3) in test tubes containing 2 mL of medium for 4h at at 30 °C. Samples were fixed with acid-alcohol mixture (glacial acetic acid : ethyl alcohol, 1 : 3 v/v) following inoculation. Pollen was considered as germinated when the pollen tube was equal to or longer than the diameter of the pollen grains. Pollen tubes were stained with lactophenol aniline-blue and aceto-orcein (Jensen 1962; Ünal 1983). Stigma/style were taken from newly opened flowers in *G. villosa.* Style were hydrolysed in 1N HCl for 10 min at 60 °C, washed in dH_2O and squashed in aceto-orcein and aniline blue prepared in lactophenol (Jensen 1962). Photographs were taken with the help of an Olympus E330 digital camera on an Olympus CX31 microscope.

Results

Androecium

The androecium of *G. villosa* was formed by six stamens. Anthers were yellow and basifixed. Mature anthers were generally 2-3 mm long, but anthers of 4 mm have been encountered. Filaments were shorter than the anthers. Microsporangia opened from stomiums by cracking in mature anthers.

Anther Wall

The anthers of *G. villosa* were tetrasporangiate. There were 3 or 4 cell lineages in young anther wall. It comprised of four layers; epidermis, endothecium, middle layer and tapetum. Layers except tapetum were preserved totally before pollen mitosis (Fig. 2a). There were two layers in mature cell wall. These were the outermost epidermis and under endothecium. Epidermis cells were quite flat compared to endothecium cells. The integrity of these cells is usually preserved (Fig. 2b). Endothecium thickenings began to develop in the phase in which microspores began to release from the tetrads. They complete their developments in mature pollen phase. Structure of young endothecium cells was simple and nuclei were large. Structure of fibrous thickenings was also simple (Fig. 2c). Nuclei of endothecium cells were quite small in mature anther walls. It was observed that fibrous thickenings displayed anastamoses (Fig. 2d). Tapetum cells were one- or two-nucleated. Two-nucleated tapetal cells were developed

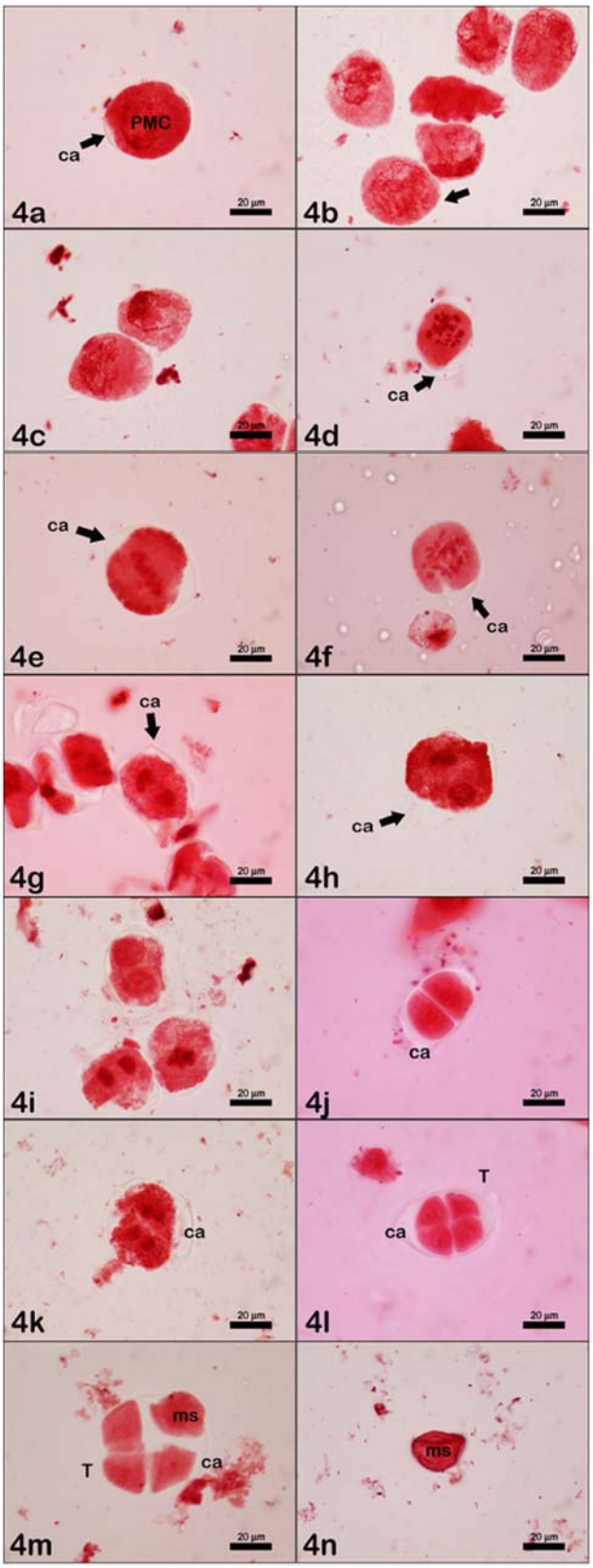

Fig. 4. Succesive type of meiosis in pollen mother cells of *G. villosa*. 4a – leptotene; 4b – zygotene (arrow); 4c – pachytene (bouquet stage); 4d – diplotene; 4e – metaphase I (side view); 4f – metaphase I (view from above); 4g – anaphase I; 4h – telophase I; 4i – pollen mother cells in different phases (dyad, metaphase I ve telophase I); 4j – dyad phase; 4k – telophase II; l, tetrad; m, microspores become free from tetrad; n, microspore (ca, callose; D, dyad; ms, microspore; PMC, pollen mother cell; T, tetrad).

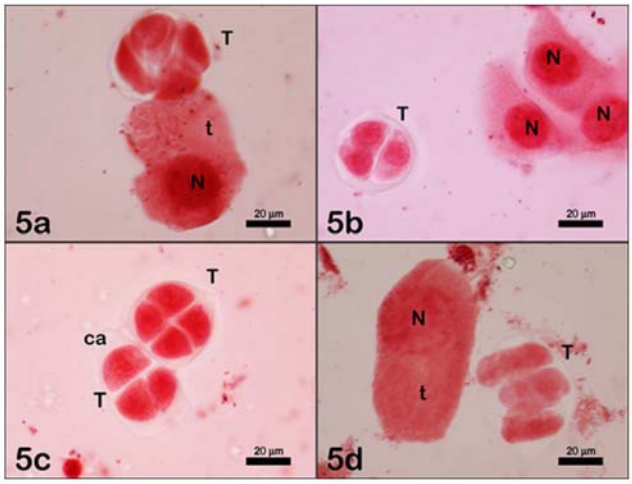

Fig. 5. Tetrad types seen in *G. villosa*. 5a – isobilateral; 5b – decussat; 5c – isobilateral and decussat tetrads; 5d – linear (ca; callose; N, nucleus; T, tetrad; t, tapetum).

at the end of normal mitosis (Fig. 3a,b). Tapetum is glandular type and when microspores released from the tetrad, they began to degenerate. Tapetum cells were degenerated totally in mature pollen phase, but rarely some remained undegenerated (Fig. 3c). Normal mitosis was generally seen in tapetum cells of *G. villosa* but micronuclei (arrow) were rarely seen in some of them (Fig. 3d).

Microsporogenesis

Some differences in the number of microspores and size of pollens in *G. villosa* were observed at the end of meiosis. Therefore phases of microsporogenesis were studied in detail. Meiosis in pollen mother cells (PMCs) was asynchronous in one anther locus of *G. villosa*. Dyads were found as well as tetrads in the same anther locus. Asynchrony between the anthers of the same bud was also found. Nuclei of PMCs in the same anther were similar in volume to each other at interphase. Callose wall was found around the PMCs. In most PMCs the course of meiosis was regular (Fig. 4a-m). Cytokinesis was of the successive type. Generally isobilateral (Fig. 5a,c) and decussate (Fig. 5b,c) types of tetrads were seen, but rarely linear type tetrads (Fig. 5d) were also seen.

Pollen Mitosis

Microspores, which became free by being released from the callose in *G. villosa* at the tetrad phase, were observed to be shell-shaped and their cell walls were seen to be wrinkled (Fig. 4m). In following phases, it was observed that microspores had been swollen such that cell walls had flattened and vacuoles had been formed at a pole. The nucleus was near the cell wall and polarity was seen in microspore (Fig. 6a,b). Prophase (Fig. 6c,d), metaphase (Fig 6e,f,g), anaphase (Fig. 6h) and telophase (Fig. 6i,j,k) were normal in pollen mitosis. At the end of pollen mitosis, it was seen that nucleus nearby cell wall had formed generative cell and

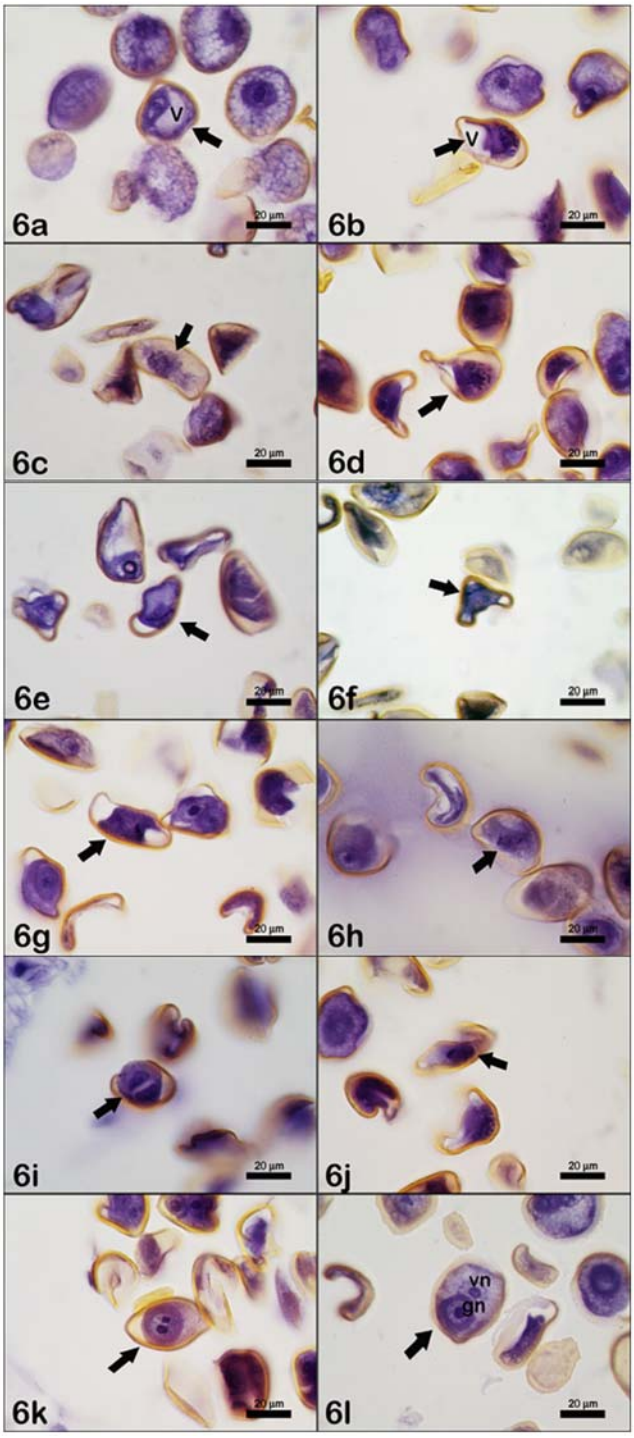

Fig. 6. Pollen mitosis in *G. villosa*. 6a,b – one-nucleated microspores, vacuolated microspore phase (arrows); 6c – prophase (arrow); 6d – late prophase early metaphase (arrow); 6e,f,g – metaphase (arrow); 6h – anaphase; 6i – early telophase (arrow); 6j – telophase (arrow); 6k – late telophase (arrow); 6l – mature pollen (arrow) (gn, generative nucleus; V, vacuole; vn, vegetative nucleus).

the other one had formed vegetative cell (Fig. 6l). Lens-shaped generative nuclei were stained dark whereas sphere-shaped vegetative nuclei were stained lightly in two-celled mature pollens (Fig. 7a). Sterile pollen grains (Fig. 7b) and smaller than the normal pollen grains (Fig. 7c,d) were also observed at the end of pollen mitosis.

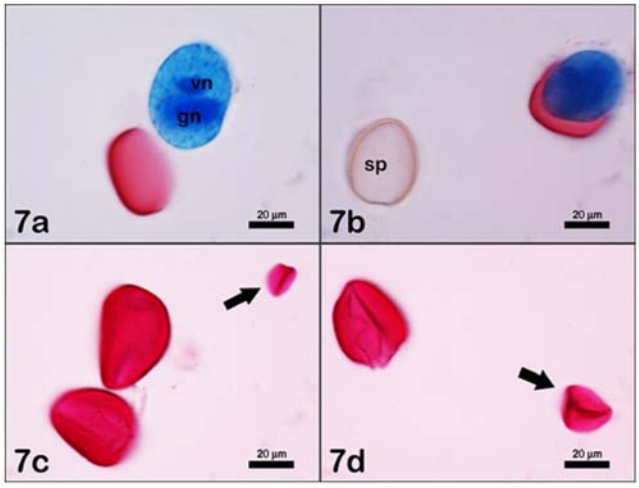

Fig. 7. Pollens of *G. villosa*. 7a – pollen grain stained with anilin blue and aceto-orcein; b – sterile pollen grain (arrow); 7c,d – small pollen grains from normal ones (arrow) (gn, generative nucleus; sp, sterile pollen; vn, vegetative nucleus).

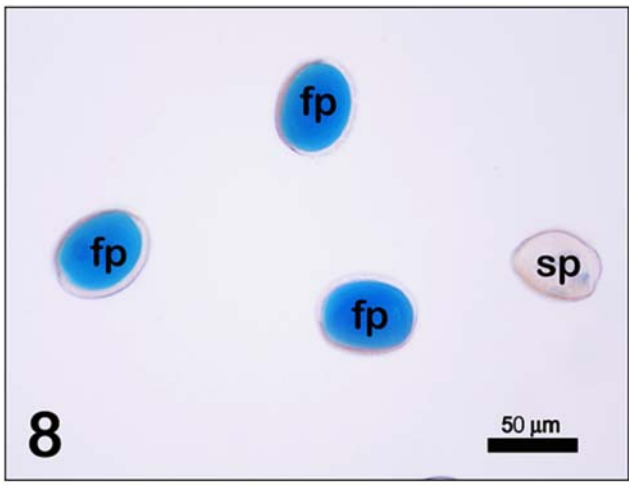

Fig. 8. Fertile and sterile pollens of *G. villosa* (fp, fertile pollen; sp, sterile pollen).

Pollen Viability

The activity of pollen in *G. villosa* was examined using light microscopy. Pollens stained with aceto/orcein and aniline blue prepared in lactophenol solution were considered to be fertile. The pollen sterility rate was found to be 24% (Fig. 8).

Microgametophyte Development

Microgametophyte development was examined *in vitro* and *in vivo* culture. Pollen grains obtained from the anthers in new blossoms (in the size of 7–12 mm) of *G. villosa* did not germinate in basic *in vitro* culture, so $Ca(NO_3)_2$, $MgSO_4$ and KNO_3 were added to the culture medium to better meet their nutritional needs. Pollen tube growth was rarely observed (Fig. 9a-d). 1000 pollen grains were counted and germination percentage of mature pollen grains was found to be 4%. During *in vitro* pollen germination of *G. villosa*, gen-

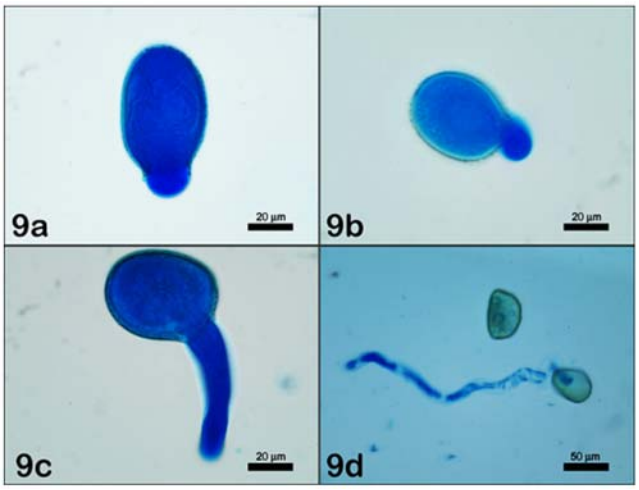

Fig. 9. *In vitro* culture of microgametophyte development in *G. villosa*. 9a – pollen grain during germination; 9b – beginning of pollen tube growth; 9c – pollen tube growth; 9d – pollen tube (pt, pollen tube).

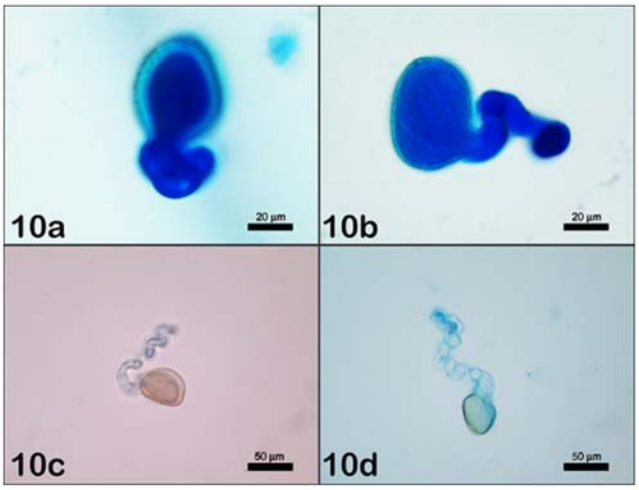

Fig. 10. Some abnormalities of microgametophyte development in *G. villosa* during *in vitro* culture. 10a,b – pollen tubes with swollen tips; 10c,d – curled and spirally growing weak pollen tubes

erally abnormal tubes were observed beside the normal ones. These abnormalities were swollen pollen tube tips (Fig. 10a,b) and weak development of some curled pollen tubes (Fig. 10c,d). Callose plug formation was not seen during *in vitro* pollen tube growth in *G. villosa*. *In vivo* pollen tube growth was also observed in *G. villosa*. Pollen tubes grew longer *in vivo* than *in vitro* (Fig. 11) and during *in vivo* pollen tube growth callose plug formation was seen in *G. villosa* (Fig. 12).

Discussion

The androecium consists of six stamens in *G. villosa*. The colours of the anthers were yellow and they were basifixed. These features were compatible with previously stated features of this species (Başak 1990). Microsporangia open from stomiums by cracking when the

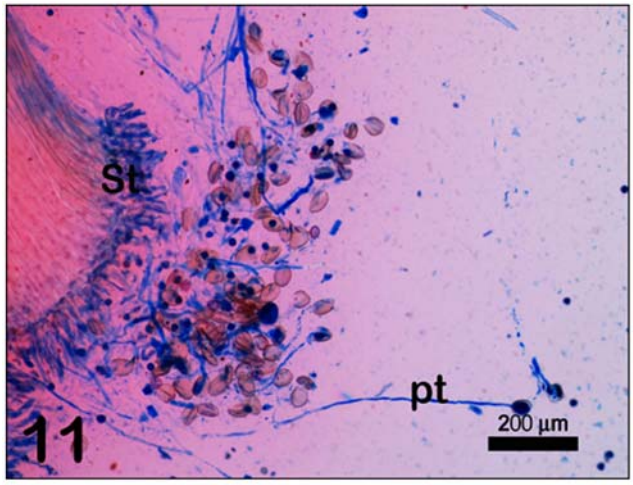

Fig. 11. *In vivo* microgametophyte development in *G. villosa* (pt, polen tube; St, stigma).

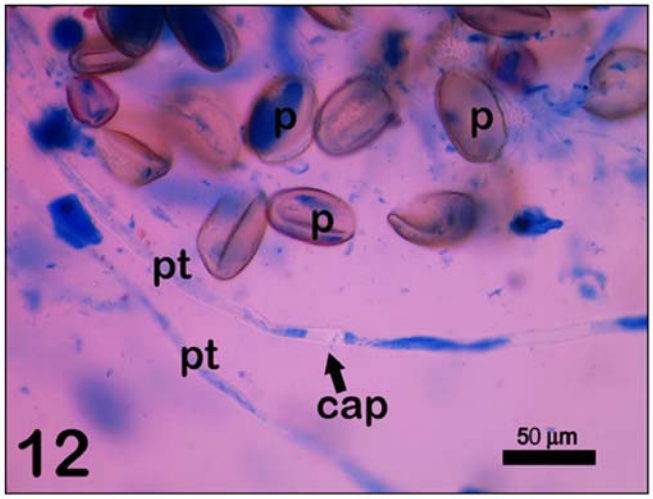

Fig. 12. *In vivo* microgametophyte development and callose plug formation in *G. villosa* (arrow) (cap, callose plug; p, pollen; pt, pollen tube).

anthers matured, as has been seen in the other members of the Liliaceae family (Watson & Dallwitz 1992).

The anthers of *G. villosa* were tetrasporangiate as in the other members of Liliaceae (Davis 1966). The young anther wall consisted of an epidermis followed to the inside by a layer of cells in the endothecium, one or two layers of cells forming the middle layer and the internal tapetum. Epidermis remained entire till the end of anther development. Permanent epidermis in the mature anther has also been reported in Araceae, Begoniaceae, Gramineae, Magnoliaceae families as well as Liliaceae (Ünal 2004).

There is a well-developed endothecium layer beneath the epidermis in the mature anther wall of *G. villosa*. Fibrous thickenings displaying anastomosis were seen in endothecium cells. Fibrous thickenings of endothecium have also been seen in *Tulipa aitchisonii* L. (Koul & Wafai 1981), *Tulipa clusiana* (L.) DC. (Wafai & Koul 1982), and *Gagea stipitata* Salisb. (Koul et al.

1976) which are also members of the Liliaceae family, and in *Leucojum aestivum* (Ekici & Dane 2012a) from Amaryllidaceae. Horseshoe-shaped thickenings of endothecium were occurred in *Bellevalia edirnensis* Özhatay & Mathew from Hyacinthaceae family (Dane 2006).

Middle layer of the anther wall in *G. villosa* consisted of one or two cell layers, as in *Gagea stipitata* (Koul et al. 1976), *Tulipa aitchisonii* (Koul & Wafai 1981) and *Tulipa clusiana* (Wafai & Koul 1982) from the same family, and it was degenerated in the mature anther. Middle layer in *Crinum defixicum* (Amaryllidaceae) consisted of four cell layers and it displays fibrous thickenings in mature anther (Davis 1966).

Tapetum of *G. villosa* was glandular type like *Gagea stipitata* (Koul et al. 1976), *Tulipa aitchisonii* (Koul & Wafai 1981), *Tulipa clusiana* (Wafai & Koul 1982) from Liliaceae. Amoeboid tapetum which was two-four layered was seen in Araceae family. It was also seen in *Tradescantia* L. (Liliaceae), *Alisma* L. (Alismataceae), *Butomus* L. (Butomaceae), *Typha* L. (Typhaceae) (Ünal 2004).

Tapetum cells of *G. villosa* had one to two nuclei. One-nucleated tapetum cells were seen in *Allium textile* (Alliaceae) (Khaleel & Mitchell 1982). Glandular tapetum cells generally become multi-nucleated but fusion of nuclei sometimes occurs in Liliaceae (Davis 1966). Tapetum cells were one– to four nucleated in *Tulipa clusiana* (Wafai & Koul 1982).

Micronuclei were seen in the cytoplasm of some tapetum cells in *G. villosa*. These were non-centromeric fragments which formed by breakage of some chromosomes during mitosis or withdrawal of some chromosomes to the poles caused by irregularities of mitotic spindles. Some irregular chromosome couplings lead to the delay in their movements toward the poles and caused them to be left in the middle of the cell. Some of these disappeared and finally microspores with no equal size of nuclei were formed. However, some of them do not disappear and form micronuclei (Silva-Stort 1984). This was seen in the tapetum cells of *L. aestivum* (Amaryllidaceae) (Ekici & Dane 2012b).

Microsporogenesis phases in PMC's of *G. villosa* were generally regular. The successive type of cytokinesis is seen in PMCs during meiosis, as in most of the monocotyledons (Furness & Rudall 1999) and the other Liliaceae members (Davis 1966). But simultaneous type cytokinesis was reported in *Asphodelus, Asphodeline, Bulbine, Eremurus, Kniphofia* and *Tofieldia* from the same family (Davis 1966). Callose membranes, which began to form in the leptoten in PMCs of *G. villosa*, dissolve just as in many angiosperms. On the other hand, in *Citrus limon* (Rutaceae) callose deposition has taken place in interphase (Horner & Lernsten1971).

Asynchronous meiosis in PMCs was seen in the anther locus of *G. villosa*. On the other hand, synchronous microsporogenesis and simultaneous cytokinesis were seen in *Habenaria willd* (Orchidaceae) (Sharma & Vij 1987). It was seen that dyads and tetrads have been found together in some anthers of *G. villosa*. Furthermore, asynchronization was also determined among the anthers of the same bud. Microspore phase could be seen in one anther whereas the tetrad phase was seen in another one. This was also identified in *Glycine max* L. Merr. (Fabaceae) (Albertsen & Palmer 1979) and *Lilium longiflorum* L. (Liliaceae) (Shull & Menzel 1977). Cytoplasmic content of all tetrads were also disrupted in some anthers of *G. villosa*. Meiotic irregularities can be originated from genetic instability and also environmental factors.

In *G. villosa* like in the other Liliaceae members, linear tetrads were rarely seen besides isobilateral and deccusate tetrads (Davis 1966). Isobilateral and tetrahedral tetrads were seen in *Tulipa clusiana* (Wafai & Koul 1982) and *Gagea stipitata* (Koul et al. 1976) from Liliaceae; on the other hand, isobilateral tetrads were seen in *Bellevalia edirnensis* (Hyacinthaceae) (Dane 2006).

Polarity was observed in pollen mitosis in *G. villosa* as in *Sternbergia lutea* Ker-Gawler ex Sprengel (Dane 1998) and *Bellevalia edirnensis* (Dane 1999) and *Leucojum aestivum* (Ekici & Dane 2012b). During polarization, nucleus migrated to the proximal pole opposite to the aperture and a big vacuole was formed in the distal pole. This is peculiar to monocotyledons. It was determined that although nucleus migrated to the proximal pole in *Cypripedium fasciculatum* (Orchidaceae) (Brown & Lemmon 1994) in pollen mitosis as in the other monocotyledons, nucleus migrates to the distal pole in *Phalenopsis* (Brown & Lemmon 1992). During pollen mitosis, vacuolization (Ekici & Dane 2004) has a role in the formation of polarization as well as nucleus migration (Brown & Lemmon 1994; Ekici & Dane 2004). According to Ünal (2004), the region which nucleus migrates during the pollen mitosis, is identified the location of generative cell. This was also supported by observations related to pollen mitosis of *G. villosa*.

Pollen, the male gametophyte of higher plants, is a biological system playing a central role in sexual plant reproduction. Pollens are formed as a result of meiosis in PMC's which were in the pollen sacs of the anther. Pollens became two-celled while mitosis was occurring in the anther (Cresti et al. 1992). Pollen mitosis of *G. villosa* was also occurred in anther and two-celled pollen grain was formed. Generative cell nuclei are long and lens-shaped whereas the vegetative cell nuclei were lobed. Mature pollen grains were two celled in *Tulipa clusiana* Wafai & Koul 1982) and *Gagea stipitata* (Koul et al. 1976), three celled in *Allium textile* (Alliaceae) (Khaleel & Mitchell 1982), and two or three celled in some of the species of the Araceae family (Davis 1966) when shed.

Pollen grains of *G. villosa* were studied by Karaca et al. (2007). They were heteropolar, oblate and their apertures were sulcate. Sulcus membranes were circular, polygonal and covered granules of different diameter. Their ornamentation was determined as reticulate to reticular crystate.

Meiotic irregularities in sporogeneous cells appear to cause spores to lose their viability. These spores can-

not grow rapidly and absorb the nutrients normally supplied by the tapetum. As a result of this, sterile pollen grains are formed. Furthermore, a small ratio of loose pollen has been seen in the fertile plants. This ratio is approximately 15% and could vary between 2% and 20% (Zenkteller 1962). On the other hand, in *G. villosa* this ratio was determined as 24% and it was determined to be higher than the others. According to Horner (1977), one of the reasons for pollen sterility is that nutrients cannot be transmitted into the microspores from the middle layer due to degeneration of the tapetal cells. The tapetum is considered the source of callose so that abnormal development of the tapetum in the PMCs can cause failure of microspore release from tetrads and, thus, further development (Horner 1977). Meiotic irregularities are the other cause of pollen sterility.

Mature pollen grains of *G. villosa* were germinated in a very low ratio *in vitro* medium. Germination percentage of mature pollen grains was identified as 4%.

Generative cells of the pollen tubes in *G. lutea* were seen 8 hours germination in the culture medium (Ignatowska & Bohdanowicz 2010) but nuclei could not be seen in pollen tubes of *G. villosa*. During the formation of pollen tubes, some differences in the behavior of the tube nucleus were observed. In two-celled pollen grains, the cytoplasm filling the grain belongs to the vegetative cell and this also forms the cytoplasm in the pollen tube. Therefore, vegetative cell is called tube cell and its nucleus is named as tube nucleus (Ünal 2004).

When the pollen tube formed, the vegetative nucleus usually moves first from the pollen grain into the tube, which has been observed in many angiosperms. During the formation of some pollen tubes, on the other hand, it was observed that both cell nuclei stayed in the pollen without moving along the pollen tube. These kinds of differences in nucleus behavior observed in the pollen tube cells could not be identified in *G. villosa.*

During *in vitro* pollen germination of *G. villosa*, some abnormal tubes were seen besides normal tubes as in *Vicia galileae* Plitm. & Zoh. (Fabaceae) (Dane & Meriç 1999). Normal pollen tubes however were rarely seen in *G. villosa*. It was observed that some pollen tubes were less developed and some of them had swollen tube tips. This has been caused by the abnormalities in the nuclei of some microspores (Ünal 2004).

Callose plug formation also could not be seen in the pollen tubes of *G. villosa* which were germinated *in vitro,* but it was seen in the *in vivo* germinated pollen tubes.

Commonly pollen tubes germinated *in vivo* were longer than *in vitro* germinated ones in *G. villosa* as in *Leucojum aestivum* L. (Ekici & Dane 2012b) and *Prunus avium* L. (Ciampolini et al. 1982). Nutrients which were secreted from stigma, style and ovary and chemotropic substances could cause longer *in vivo* pollen tubes (Tilton & Horner 1980; Tilton et al. 1984). Growth of *in vivo* pollen tubes includes two phases. The first phase depends totally on pollen and contents of the pollen tube and depends on stylar characteristics. The second phase in contrast relies on ovary characteristics and does not occure *in vitro* germination. Styles in which pollen tube growth occurred were dissected from ovaries in the studies done with *Nicotiana alata* L. and *Petunia hybrida* Hort. It was seen that pollen tubes did not reach the end of the style and tube growth ended at approximately 1/3 to 2/3 of the length of the style in those plants. Pollen tubes however reached the inside of ovaries that were not dissected from the style. This mechanism could not be explained yet (Bergamini-Mulcahy & Mulcahy 1985).

In conclusion some cytological and embryological characteristics of *G. villosa* are studied for the first time. Generally, development of male gametophyte is normal. The data gained from this study may contribute to embryological characteristics which were used in taxonomy of Liliaceae.

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References

Albertsen M.C. & Palmer R.G. 1979. A comperative light and electron-microscopic study of microsporogenesis in male sterile (MS1) and male fertile soybeans. (*Glycine max* (L.) Merr.). Am. J. Bot. **66:** 253–265.

Alexander M.P. 1969. Differential staining of aborted and non-aborted pollen. Stain Tech. **44:** 117–122.

Başak N. 1990. Cytotaxonomical Researches on Liliaceae (*Fritallaria, Tulipa, Gagea*) Family in Edirne and Kırklareli Region. Edirne. PhD Thesis, Edirne, pp. 62–95.

Bergamini-Mulcahy G. & Mulcahy D.L. 1985. Ovarian influence on pollen tube growth, as indicated by the *semivivo* technique. Am. J. Bot. **72:** 1078–1080.

Brown R.C. & Lemmon B.E. 1992. Pollen development in orchids 4. Cytoskeleton and ultrastructure of the unequal pollen mitosis in *Phalaenopsis*. Protoplasma **167:** 183–192.

Brown R.C. & Lemmon B.E. 1994. Pollen mitosis in the slipper orchid *Cypripedium fasciculatum*. Sex. Plant Reprod.**7:** 87–94.

Caparelli K.F., Peruzzi L. & Cesca G. 2006. A comparative analysis of embryo-sac development in three closely-related *Gagea* species (Liliaceae), with some considerations on their reproductive strategies. Plant Biosyst. **140:** 115–122.

Ciampolini F., Cresti H. & Kapil R.N. 1982. Germination of cherry pollen grains *in vitro* – An ultrastructural study. Phytomorphology **32:** 364–373.

Cresti M., Blackmore S. & Van Went J.L. 1992. Atlas of Sexual Reproduction in Flowering Plants. Springer-Verlag, Berlin, 14 pp.

Dane F. 1998. Mitotic divisions in root tip cells and pollen grains of *Sternbergia lutea* (L.) Ker-Gawl. Ex Sprengel (Amaryllidaceae). MÜFBED. **14:** 1–10.

Dane F. 1999. A Study of pollen mitosis and pollen morphology in hexaploid (2n=24) *Bellevalia edirnensis* Özhatay & Mathew. Turk. J. Biol. **23:** 357–368.

Dane. F. 2006. Cytological and embryological studies of *Bellevalia edirnensis* Özhatay & Mathew (Hyacinthaceae). Acta Bot. Hung. **57:** 339–354.

Dane F. & Meriç Ç. 1999. Reproduction biology of *Vicia* L. II. Cytological and cytoembryological studies on the anther wall, microsporogenesis, pollen mitosis and development of the male gametophyte of *Vicia galileae* Plitm. & Zoh. Turk. J. Biol. **23:** 269–281.

Davis G.L. 1966. Systematic Embryology of the Angiosperms. John Wiley & Sons Inc, New York, pp. 157–160.

Ekici N. & Dane F. 2004. Polarity during sporogenesis and gametogenesis in plants. Biologia **59:** 687–696.

Ekici N. & Dane F. 2012a. Some histochemical features of anther wall of *Leucojum aestivum* (Amaryllidaceae) during pollen development. Biologia **67:** 857–866.

Ekici N. & Dane F. 2012b. Microsporogenesis, pollen mitosis and *in vitro* pollen tube growth in *Leucojum aestivum* (Amaryllidaceae), pp. 173–190. In: Mworia J. (ed.), Botany, Intech, Rijeka.

Furness C.A. & Rudall P.J. 1999. Microsporogenesis in monocotyledons. Ann. Bot. **84:** 475–499.

Greilhube J., Ebert I., Lorenz A. & Vyskot B. 2000. Origin of facultative heterochromatin in the endosperm of *Gagea lutea* (Liliaceae). Protoplasma **212:** 217–226.

Horner Jr. H.T. 1977. A Comparative light- and electron-microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile sunflower (*Helianthus annuus*). Am. J. Bot. **64:** 745–759.

Horner Jr. H.T. & Lernsten N.R. 1971. Microsporogenesis in *Citrus limon* (Rutaceae). Am. J. Bot. **58:** 72–79.

Ignatowska M. & Bohdanowicz J. 2010. Ultrastructure and immunocytochemistry of dimorphic sperm cells in pollen tubes of *Gagea lutea* (L.) Ker-Gawl. In: Abstracts of the XXIX Conference on Embryology-Plants-Animals-Humans, Poland. Acta Biol. Cracov. Bot. **52 (suppl.1):** 62.

Jensen W.A. 1962. Botanical Histochemistry. W. H. Freeman and Company, San Francisco, pp. 203–204.

Johansen D.A. 1940. Plant Microtechnique. Mc Graw-Hill Book Company, New York & London, 52 pp.

Joshi A.C. 1946. Development of the embriyo-sac of *Gagea fascicularis* Salisb. Bull. Torrey Bot. Club.**67:** 155–158.

Karaca Z., Yaşar A., Vural E. & Vural C. 2007. Pollen morphology of certain geophyte plants (Liliaceae, Iridaceae) in mount Erciyes, Kayseri. Erciyes Üniv. Fen Bil. Enst. Der. **23:** 37–46.

Khaleel T.F. & Mitchell B.B. 1982. Cytoembryology of *Allium textile* Nels. and Macbr. Am. J. Bot. **69:** 950–956.

Koul A.K. & Wafai B.A. 1981. Analysis of breeding systems in the genus *Tulipa*. I. Sporogenesis, gametogenesis and embryogeny in diploid *T. aitchisonii*. Phytomorphology **31:** 11–17.

Koul A.K., Wafai B.A. & Khan A.U. 1969. Studies on the Genus *Gagea*. II. Embryology of diploid *G. kashmirensis* Turril. Kash. Sci. **6:** 23–32.

Koul A.K., Wafai B.A. & Wakhlu A.K. 1976. Studies on the genus *Gagea*. III. Sporogenesis, early embryogeny and endosperm development in hexaploid *Gagea stipitata*. Phytomorphology**26:** 255–263.

Koul A.K. & Wakhlu A.K. 1985. Studies on the genus *Gagea* (5). Embryology of *Gagea reticulata* Schult. J. Jpn. Bot. **60:** 361–369.

Nemec B. 1912. Über die befruchtung bei *Gagea*. Bull. Inter. Acad. Sci. Boheme **17:** 160–176.

Özhatay N. 2002. Diversity of bulbous monocots in Turkey with special reference. chromosome numbers. Pure Appl. Chem.**74:** 547–555.

Peterson A., John H., Koch E. & Peterson J. 2004. A molecular phylogeny of the genus *Gagea* (Liliaceae) in Germany inferred from non-coding chloroplast and nuclear DNA sequences. Plant. Syst. Evol. **245:** 145–162.

Romanov I.D. 1936. Die embryosackentwicklung in der gattung *Gagea* Salisb. Planta **25:** 438–459.

Romanov I.D. 1961. The origin of the unique structure of endosperm nuclei in *Gagea*. Dokl. Bot. Sci. Sect. **141:** 188–190.

Saddiqi S.A. & Hashmi M.S. 1975. The development of female gametophyte in *Gagea persica* Boiss. Geobios **2:** 87–88.

Sharma M. & Vij S.P. 1987. Embryological studies in orchidaceae VI: *Habenaria* willd. Phytomorphology **37:** 327–335.

Shull J.K. & Menzel M.Y. 1977. A study of reliability of synchrony in the development of pollen mother cells of *Lilium longiflorum* at the first meiotic prophase. Am. J. Bot. **64:** 670–679.

Silva-Stort M.N. 1984. Sterility barriers of some artificial F1 orchid hybrids. I. microsporogenesis and pollen germination. Am. J. Bot. **71:** 309–318.

Stenar H. 1927. Über die entwicklung des siebenkernigen embryosackes bei *Gagea lutea* Ker. nebst einigen bemerkungen über die reduktionsteilung bei *Gagea minima* Ker. Svensk. Bot. Tidskr. **21:** 344–360.

Tilton V.R. & Horner H.T. 1980. Stigma, style and obturator of *Ornithogalum caudatum* (Liliaceae) and their function in the reproductive process. Am. J. Bot. **67:** 1113–1131.

Tilton V.R., Wilcox L.W., Palmer R.G. & Albertsen M.C. 1984. Stigma, style and obturator of soybean, *Glycine max* (L.) Merr (Leguminosae) and their function in the reproductive process. Am. J. Bot. **71:** 676–686.

Ünal M. 1983. Correletion between pollen stainability and pollen viability. Nature Series A **7:** 572–574.

Ünal M. 2004. Plant (Angiospermae) Embryology. Marmara University, Faculty of Science and Arts, Press No: 11. Mart Press, Ýstanbul, pp. 24-28.

Vardar F., İsmailoğlu I. & Ünal M. 2012. Embryological and cytological features of *Gagea bohemica* (Liliaceae). Turk. J. Bot. **36:** 462–472.

Wafai B.A. & Koul A.K. 1982. Analysis of breeding systems in *Tulipa*. II. sporogenesis, gametogenesis and embryogeny in tetraploid *T. clusiana*. Phytomorphology **32:** 289–301.

Watson L. & Dallwitz M.J. 1992. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 19th October 2013. http://delta-intkey.com'. 1992 onwards. (accessed 15.01.2014)

Zarrei M., Zarre S., Wilkin P. & Rix M. 2007. Systematic revision of the genus *Gagea* Salisb. (Liliaceae) in Iran. Bot. J. Linn. Soc. **154:** 559–588.

Zenkteler M. 1962. Microsporogenesis and tapetal development in normal and male-sterile carrots (*Daucus carota*). Am. J. Bot. **49:** 341–348.

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