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Microsporogenesis, Pollen Mitosis and *In Vitro* Pollen Tube Growth in *Leucojum aestivum* (Amaryllidaceae)

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1. Introduction

The full range of gene expression leading to male gamete formation in flowering plants begins with determination of the stamen whorl in flower development and ends with release of mature sperm into the embryo sac near the egg and central cell (Bhojwani & Soh, 2001). This study focused on events from meiosis to pollen tube growth. Microsporogenesis has proved to be a highly informative character at the family level in angiosperm systematics, especially in monocots (Furness & Rudall, 1999). The two primary types of microsporogenesis – simultaneous and successive – differ in the relative timing of meiosis II, though intermediate conditions have been reported in some species. In the successive type, a callose wall is deposited after Meiosis I, thus forming a distinct dyad stage before the onset of Meiosis II; the resulting tetrads are predominantly tetragonal, decussate or occasionally T-shaped. In the simultaneous type, the two meioses proceed without interruption; cytoplasmic cleavage and deposition of the callose walls occur subsequently. With this type, tetrad shape is typically tetrahedral, though variable, ranging from tetragonal to tetrahedral (symmetric or asymmetric) or rhomboidal (Nadot et al., 2006). Among angiosperms, microsporogenesis seems to be more diverse within early-divergent lineages, possibly because the mechanisms controlling the process are more labile in these groups (Furness et al., 2002; Sajo et al., 2009). The asymmetric division of the microspore apparently activates a divergence of cellular programs to be initiated in the generative and vegetative cells (Park et al., 1998). Generative cell divides to form two sperm. When pollen grains are released from the anther, they are either bicellular, with one vegetative cell and one generative cell, or tricellular, with one vegetative cell or two sperm cells, depending on timing of generative cell division (Bhojwani & Soh, 2001). Pollen the male gametophyte of higher plants, is a biological system playing a central role in sexual plant reproduction (Cresti et al., 1992). Upon emergence from pollen grain, the pollen tube has to traverse distance often thousands times the diameter of the pollen grain to deliver the male gametes to the embryo sac for fertilization. The pollen tube growth pathway begins with pollen germination *in vitro* or *in vivo* (Cheung, 1996).

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This study presents new observations on microsporogenesis and pollen mitosis and pollen tube growth *in vitro* in Amaryllidaceae, in the context of an examination of cytological characters aimed at better understanding of relationships within Amaryllidaceae and other species. Previous investigations on these aspects are limited in Amaryllidaceae. According to recently records, Amaryllidaceae family has 60 genera and 800 species (Watson & Dallwitz, 2005). In recent years, *Leucojum* genus is represented by only two species (http://www.amaryllidaceae.org/Leucojum/). These are *L. aestivum* and *L. vernum*. The species that is widespread in Turkey and Thrace is *L. aestivum* subsp. *Pulchellum* (Davis, 1984). Embryological characteristics belong to this family have been gained from studies done with limited numbers of species (Şahin, 1997; Dane, 1998) and several characteristics of this family are inconsistent. It is very important to know about embryological characteristics of *L. aestivum* in terms of phylogeny. We aimed better understanding of the taxonomical characteristics of Amaryllidaceae family with this study. *L. aestivum* is also an economically important plant for galanthamin production (Heinrich, 2004) that exhibits low seed production. Microsporogenesis, pollen mitosis and pollen tube growth were investigated to understand the cause of low sexual reproductivity.

2. Material and methods

In this study, anthers of *L. aestivum* in various lengths were used. Materials were collected from Edirne Tavuk Forest in 2004-2005. Anthers were embedded in parafine and stained in Delafield's hematoxylene (Johansen, 1940). Different phases of microsporogenesis were investigated by using 1% aceto-orcein with squash preperation method. Pollen viability is studied with anilin blue prepared in lacto-phenol (Jensen, 1962). For pollen germination, the pollen grains from dehisced anthers were incubated in liquid germination medium [10% sucrose ($C_{12}H_{22}O_{11}$), 0.01% boric acid (H_3BO_3), 0.03% calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$)] in test tubes containing 2 ml of medium for 4h at room temperature. Samples were fixed with acid-alcohol mixture (1 glacial acetic acid : 3 ethyl alcohol) following inoculation. Measuring 30 randomly selected zones on slides for 3000 pollen grains determined the *in vitro* germination percentages. 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 anilin-blue and aceto orcein (Ünal, 1986). Photographs were taken with the help of Olymphus photomicroscope.

3. Results

In this study, microsporogenesis and pollen mitosis and *in vitro* pollen tube growth are described in *Leucojum aestivum* L. *(Amaryllidaceae*).

3.1 Microsporogenesis

The young anther is bilocular and tetrasporangiate; the wall comprises four layers; epidermis; fibrous endothecium; 1-2 middle layers which disappears early and a multinucleate glandular tapetum (Plate 1). Tapetum layer degenerates in mature anthers of *L. aestivum* (Plate 2). In most microspore mother cells, the course of meiosis is regular (Plate 3). Cytokinesis is of the succesive type. Isobilateral, decussat, linear, and T-shaped tetrads were seen (Plate 4). In some cells irregularities were observed (Plate 5, Table 1), including chromosome bridges and lagging chromosomes.

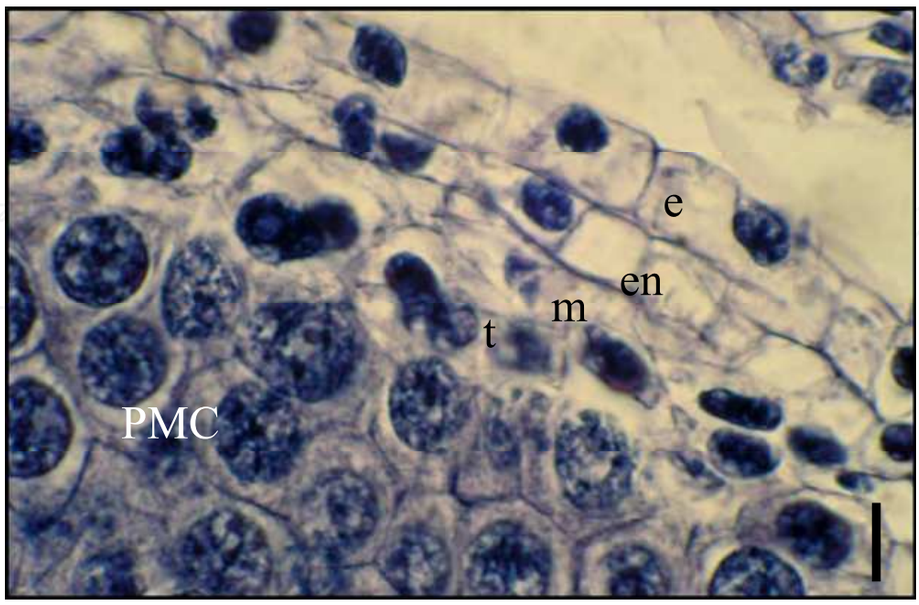


Plate 1. Cross section of young anther in *L. aestivum*; bar=20µm (e, epidermis; en, endothecium; m, middle layer; PMC, pollen mother cell; t, tapetum)

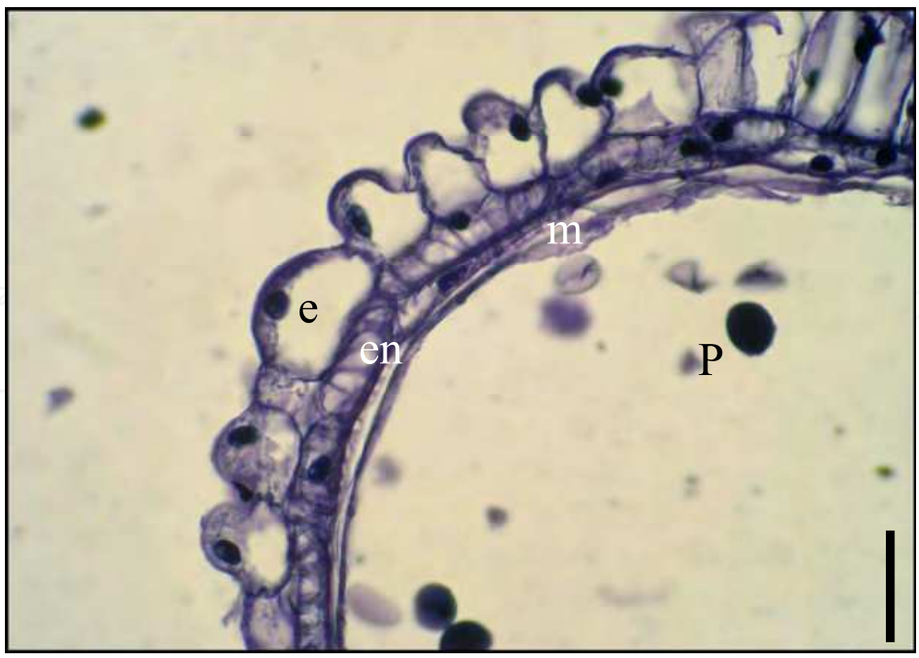
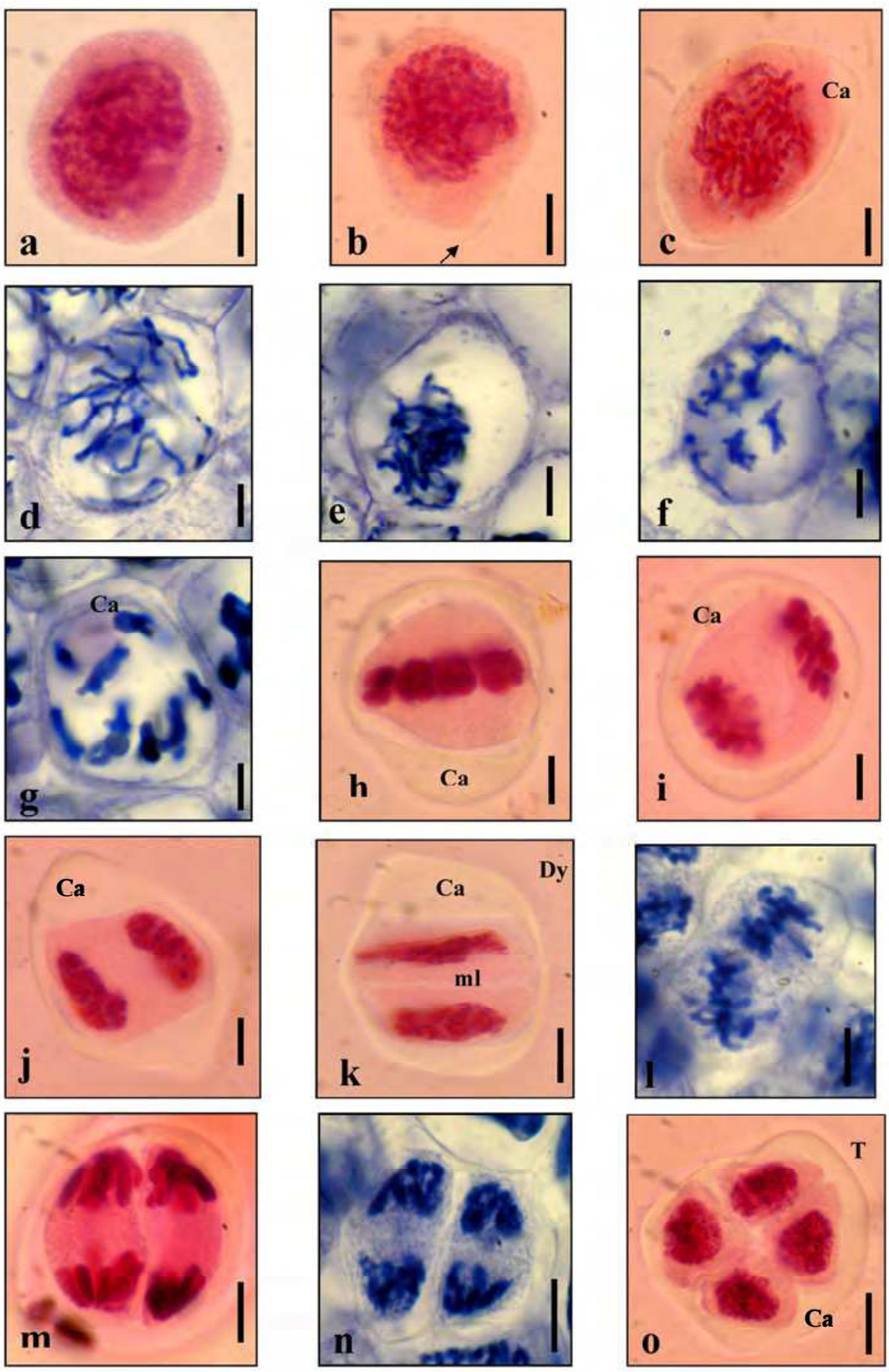


Plate 2. Cross section of mature anther in *L. aestivum*; bar=50µm (e, epidermis; en, endothecium; m, middle layer; P, pollen)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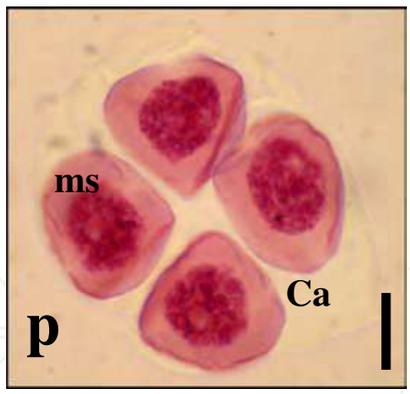

Plate 3. Successive type meiosis in pollen mother cells of *L. aestivum*. a, interphase; b,c leptotene, beginning of callose formation (arrow); d, zygotene; e, pachytene, chromosomes arranged like bouquet; f, diplotene; g, diakinesis; h, metaphase I; i, anaphase I; j, telophase I; k, dyad (interphase); k, prophase II; l, metaphase II; m, anaphase II; n, telophase II; o, tetrad; p, microspores. Bars=10µm (Dy, dyad; Ca, callose; ml, middle lamella; ms, microspore; T, tetrad)

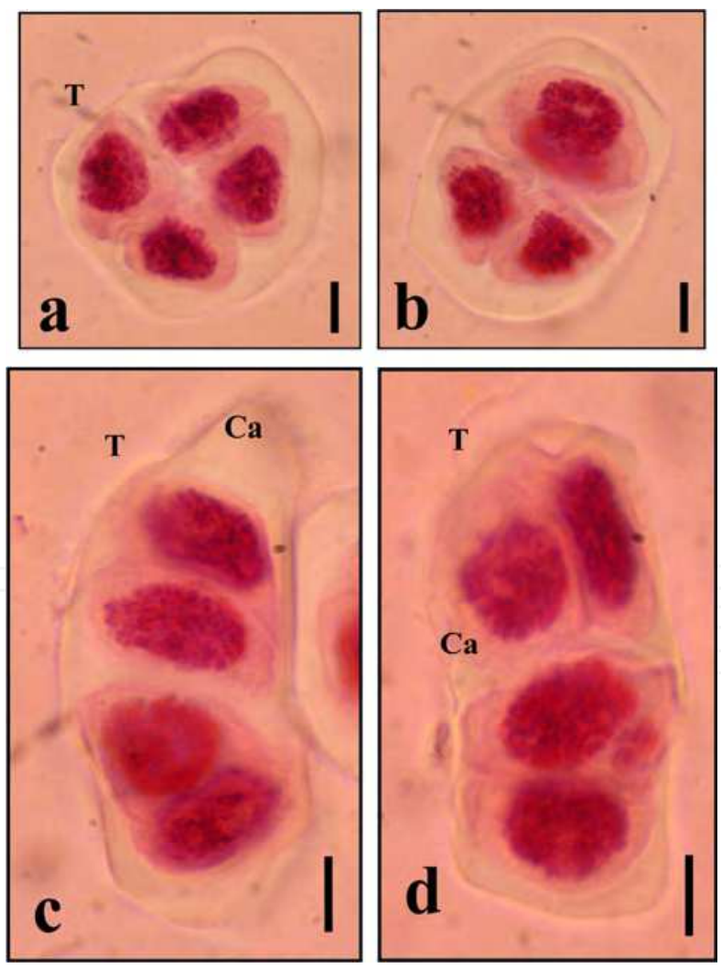

Plate 4. Tetrad types seen in *L. aestivum*; a, isobilateral; b, decussat; c, linear; d, T-shaped Bars=10µm (Ca, callose; T, tetrad)

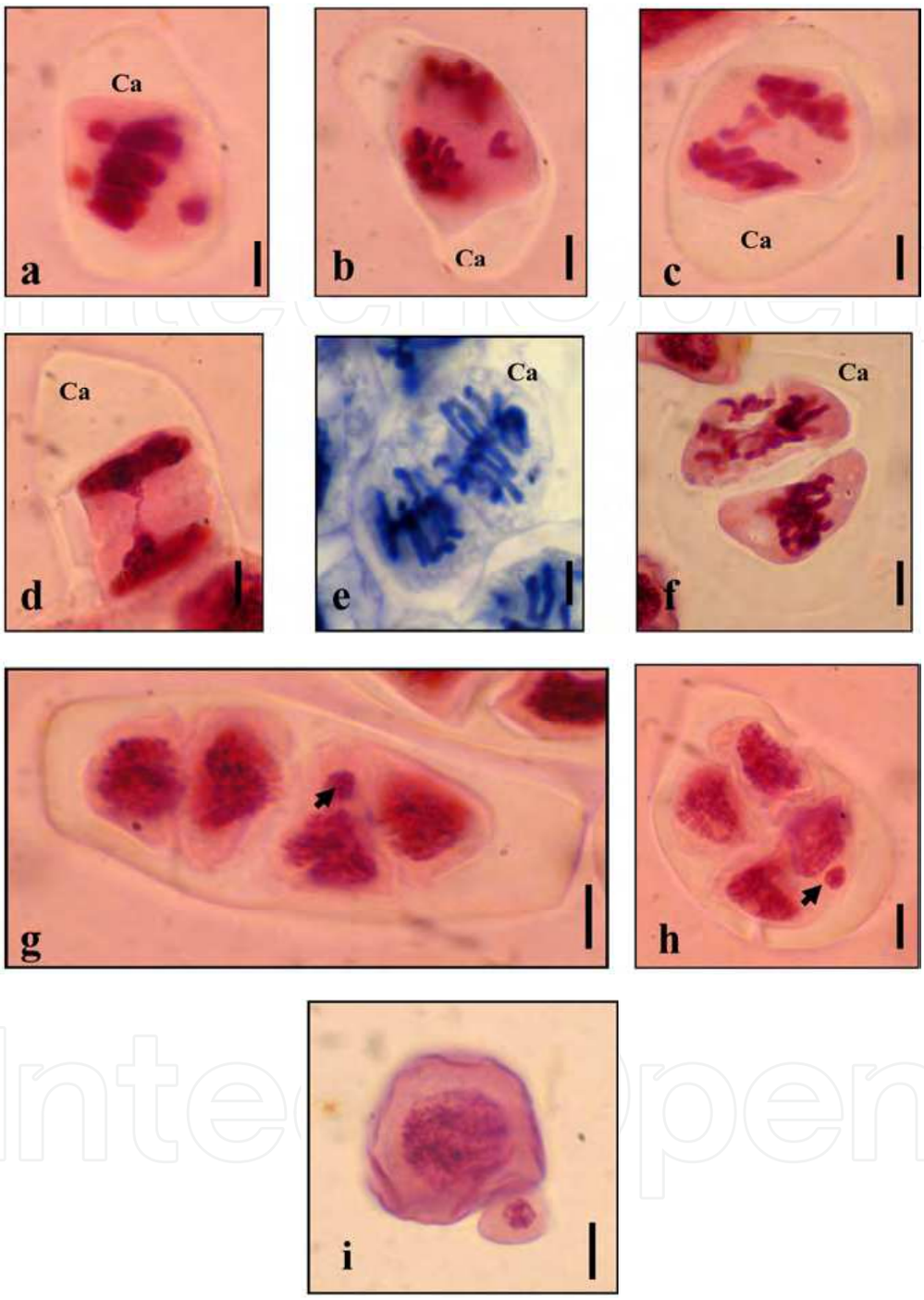

Plate 5. Some abnormalities observed in *L. aestivum* during microsporogenesis. a, chromosomes which were not arranged on equatorial plate in metaphase I; b, laggard chromosome in anaphase I; c, chromosome bridges in anaphase I; d, chromatid bridge in telophase I; e, laggard chromosome in metaphase II; f, irregularity on equatorial plate in metaphase II; g,h, micronuclei (arrows) in tetrad phase; i, normal and abnormal microspores including nucleus of various sizes. Bars=10µm (Ca, callose)

	Prophase I	Metaphase I	Anaphase I	Telophase I / Diad	Metaphase II	Anaphase II	Telophase II/ Tetrad	Total
Number of cell counted	228	112	28	176	100	68	308	1000
Number of normal cell	228	84	20	176	84	60	228	920
Number of abnormal cell	---	28	8	---	16	8	20	80
Percentage (%) of abnormal cell	0	25	28.5	0	20	11	6.4	8

Table 1. The percentage of abnormal cells observed in PMC of *L. aestivum* during meiosis.

3.2 Pollen mitosis

Microspores, which became free by being disrupted the callose in *L. aestivum* at the tetrad phase, were observed to be like a shell-shaped and their cell walls were seen wrinkled (Plate 6a). In the following phases, it was observed that microspores had been swollen that cell wall had flattened (Plate 6b) and vacuole had been formed on a pole (Plate 6c). Metaphase, anaphase and telophase were normal in pollen mitosis (Plate 6d-g). At the end of pollen mitosis, it was seen that nucleus nearby cell wall had formed generative cell and the other one had formed vegetative cell (Plate 6h,i). The pollen grains are 2 celled when shed (Plate 6j) but some abnormal pollen grains are seen (Plate 7a-c). Sterile pollen grains are also observed (Plate 8,9).

3.3 Pollen viability

The activity of pollens in *L. aestivum* was examined under light (Plate 8a,b) and flourescence microscope (Plate 9), they were usually stained well (Plate 8a). Pollens stained with aniline blue (Merck) and aniline blue (Plate 8b) prepared in Lactophenol solution were considered to be fertile. 17216 pollens were counted and the pollen sterility rate was found to be 1.7 %. The mean length and width values for pollen were determined 38.85±2.26µm and 24.36±2.1µm respectively by measuring the length of 100 pollens. Pollen grains are bilaterally symmetrical, monocolpate, prolate. The pollen grains are 2-celled when shed. The nucleus of the generative cell is long and lens-shaped and vegetative cell nucleus is lobulated.

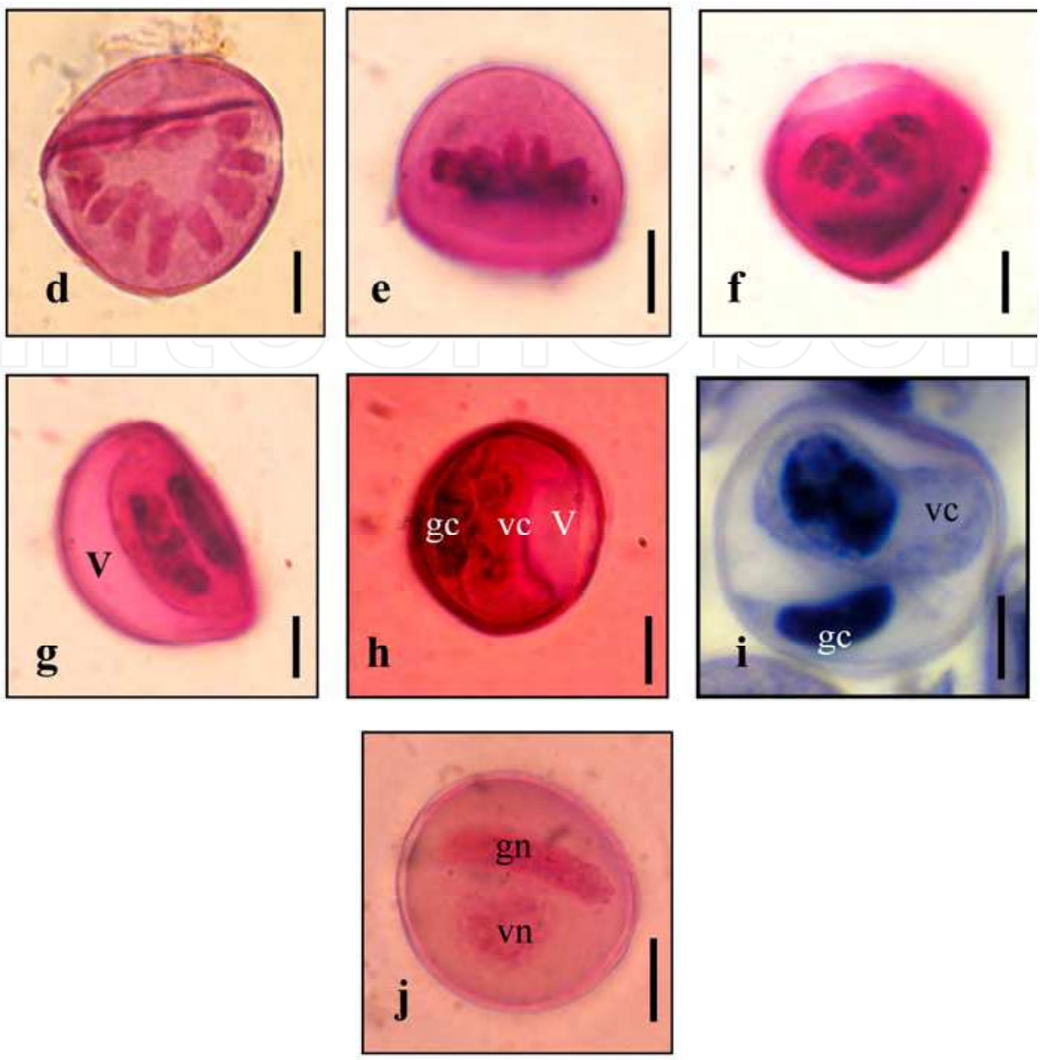

Plate 6. Pollen mitosis in *L. aestivum*. a, wrinkled microspore; b, swollen microspore; c, prophase in polarized microspore with vacuole; d,e metaphase; f, anaphase; g, telophase; h, pollen with vacuoles; i,j, mature pollen. Bars=10µm (gc, generative cell; gn, generative nucleus; N, nucleus; V, vacuole; vc, vegetative cell; vn, vegetative nucleus)

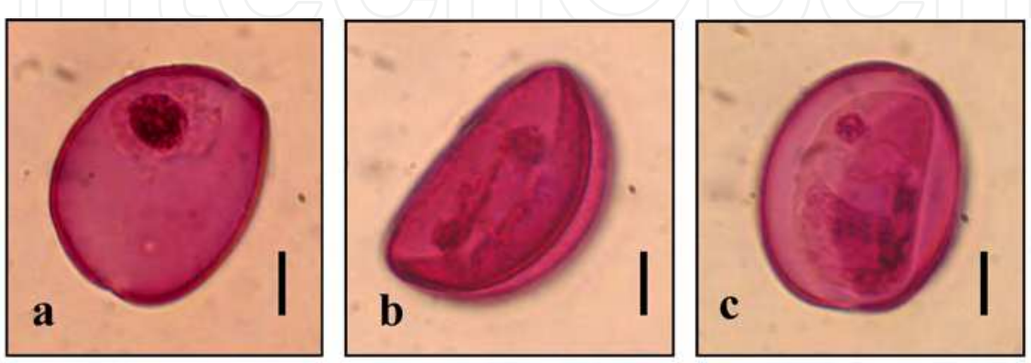

Plate 7. Some abnormalities in pollens of *L. aestivum*. Bars=10µm

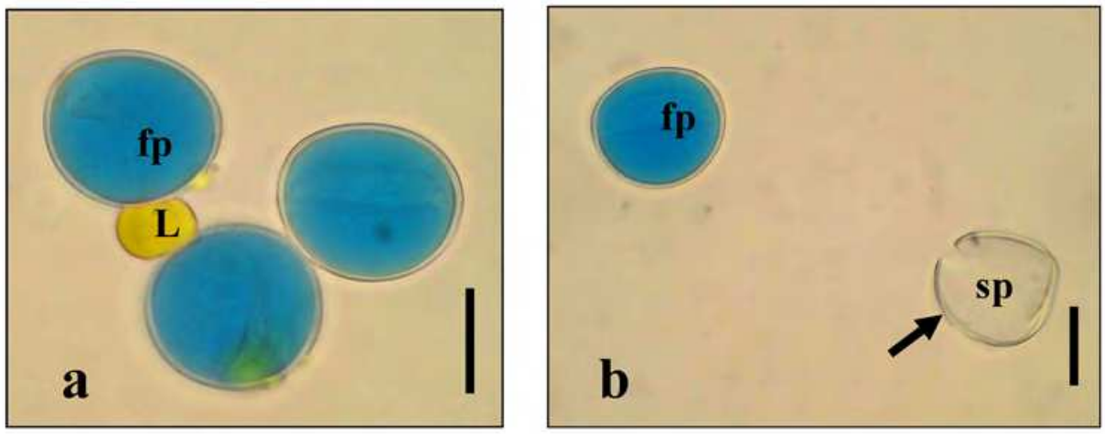

Plate 8. Mature pollen grains of *L. aestivum*; a, fertile pollens stained with aniline blue, b, fertile and sterile (arrow) pollens, Bars=20µm (sp, sterile pollen; fp, fertile pollen; L, lipid)

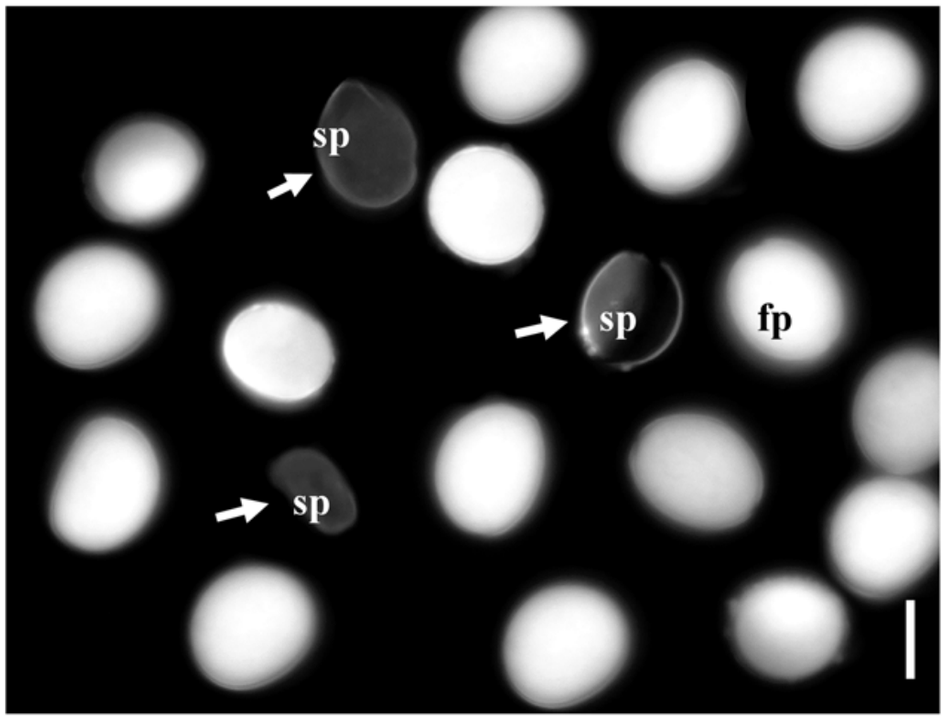

Plate 9. Fertile and sterile (arrows) pollens of *L. aestivum* under the flourescence microscope. Bar= 20µm (sp, sterile pollen; fp, fertile pollen)

3.4 Microgametophyte development

Microgametophyte development was examined in *in vitro* culture. Pollen grains obtained from the anthers in new blossoms (in the size of 15-20mm) of *L. aestivum* were germinated *in vitro* (Plate 10). 1000 pollens were counted and germination percentage of mature pollen grains was found to be 73%. Certain differences in terms of nucleus behaviors were found when germinated pollen tubes were cytological examined. In some pollen tubes it was

observed that generative and vegetative nuclei did not move in tube and stayed in pollen (Plate 11a,b). In some others, however, it was observed that both nuclei move in pollen tube.

Normally, in some of these pollen tubes, vegetative nucleus moves ahead (Plate 10d), in some others generative nucleus moves ahead (Plate 11c,d). During *in vitro* pollen germination of *L. aestivum*, abnormal tubes were observed as well as normal ones. These abnormalities were weak development of some pollen tubes (Plate 11e,f) and swollen pollen tube tips (Plate 11g-k). Callose plug formation was not seen *in vitro* pollen tube growth in *L. aestivum*. *In vitro* pollen tube growth was also observed with fluorescence microscope (Plate 12).

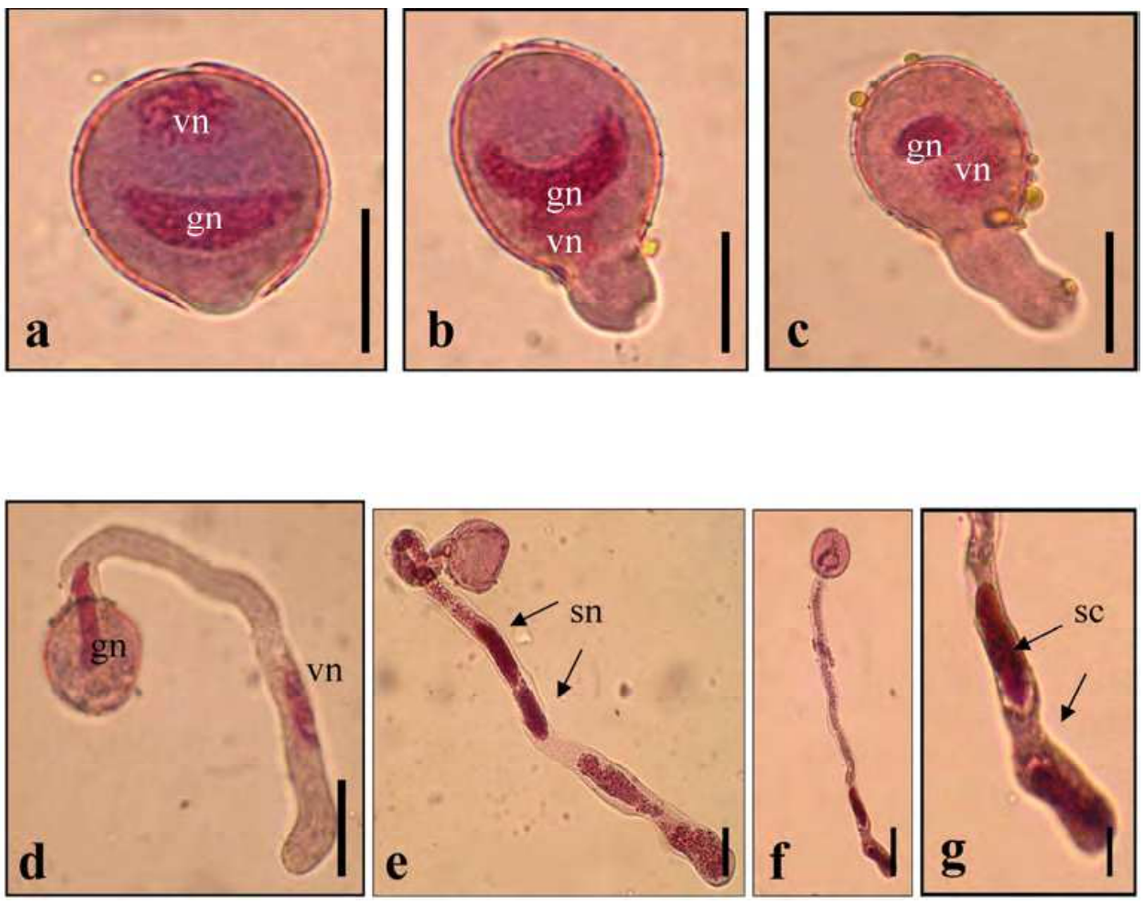

Plate 10. *In vitro* culture of microgametophyte development. a, pollen grain with two cells during germination Bar=20µm; b,c, vegetative and generative cell nuclei moving towards pollen tube, Bars=20µm; d, vegetative cell nucleus moving in prolonged tube Bar=20µm, e, sperm nuclei in pollen tube Bar=20µm; f,g, sperm cells at the tip of pollen tube, f, Bar=30µm; g, Bar=10µm (gn, generative nucleus; sc, sperm cell; sn sperm nucleus; vn, vegetative nucleus)


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Plate 11. Some abnormalities of microgametophyte development during *in vitro* culture. a,b, pollen tubes without generative and vegetative nuclei in tube; c,d, pollen tubes in which generative nuclei went forward; e,f, weak development of pollen tubes; g-k, pollen tubes with swollen tips. a-d, f, g-k, Bar=20µm; e, Bar=30µm. (gn, generative nucleus; vn vegetative nucleus)

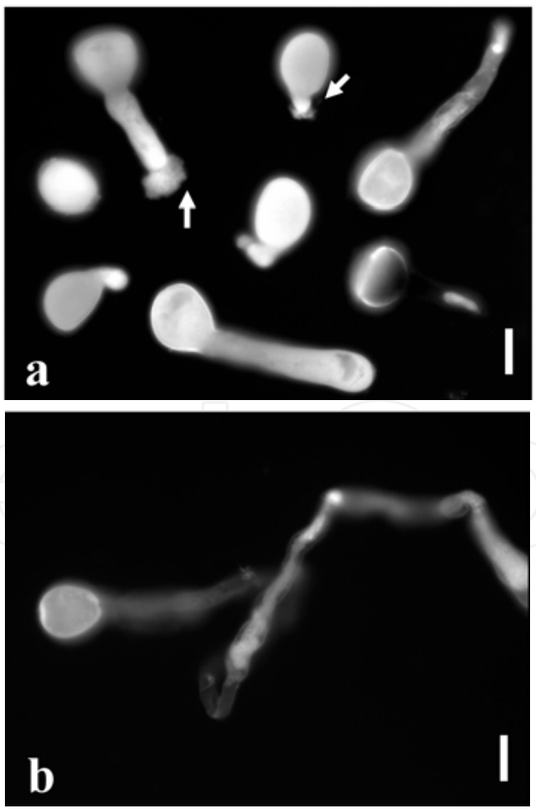

Plate 12. *In vitro* germination of pollen tubes viewed under fluorescence microscope a, developing pollen tubes and swollen tips of some pollen tubes (arrows); b, prolonged pollen tube; Bar=20µm.

4. Discussion

The anthers of *L. aestivum* are tetrasporangiate as in the other members of Amaryllidaceae. Its tapetum was with 1-2 cell lines and was glandular type like *Crinum* (Davis, 1966). Ameboid type of tapetum have been seen in *Galanthus* (Pankow 1958) that closely related with *Leucojum*. Microsporogenesis was observed to be generally regular in PMC of *L. aestivum*. Succesive type of cytokinesis is seen in PMCs during meiosis as in most of the monocotyledons (Furness & Rudall, 1999) and the other Amaryllidaceae members (Davis, 1966). Callose membrane, which began to form in the leptoten in PMCs of *L. aestivum*, dissolves just as in many angiosperms. On the other hand, in *Citrus limon* (Rutaceae) (Horner & Lernsten, 1971) callose deposition has taken place in interphase. Asynchronous meiosis in PMCs was seen in the anther locus of *L. aestivum*. On the other hand, synchronous microsporogenesis and simultaneous cytokinesis were seen in *Habenaria willd* (Orchidaceae) (Sharma & Vij, 1987). It was observed that PMCs at the top of some anthers in *L. aestivum* were in zygotene, whereas the ones at the bottom of the anther were in the pachytene. The phases at the top and the bottom of the anther were monitored to be far more different in advanced phases and the diakinesis stage was seen at the top, dyads were seen at the middle and tetrads were seen at the bottom of the anther. Furthermore, asynchronization was also determined among the anthers of the same bud. Microspore phase could be seen in one anther while tetrad phase was seen in another one. This was also identified in *Glicine max* (L.) Merr. (Fabaceae) (Albertsen & Palmer, 1979) and *Lilium longiflorum* L. (Liliaceae) (Shull & Menzel, 1977). Gradual transmitting of the nutrition from the bottom to the top can be the cause of asynchronization in the same locus of the anthers in *L. aestivum* and other plants. It was seen that meiosis I had usually been synchronous in the anther locus of *Coccina indica* aut. Non. (L.) Cogn., *Momordica charantia* Linn. and *Melothria maderaspatana* (L.) Cogn. (Cucurbitaceae) (Desphande et al., 1986); however, synchronization in meiosis II had become disrupted. This was explained by the existence of cytoplasmic channels in the early phases of meiosis I. It was seen in the ultrastructure of anther membrane of *L. aestivum* between the sporogeneous tissue cells. Then, in leptotene, cytoplasmic channels between PMCs have disappeared with the formation of callose membrane.

In this study, some irregularities were also seen although microsporogenesis in *L. aestivum* was generally regular. It was seen that the chromosomes had not been normally arranged in the equatorial plate in metaphase in some cells, lagging chromosomes were observed in anaphase I and telophase I and some chromosomes had formed choromosome bridges and there were micronuclei in tetrad and microspore stages. During anaphase II, micronuclei were formed by lagging chromosomes in *Gagea stipitata* (Liliaceae) (Koul et al., 1976), *Tulipa clusiana* (Liliaceae) (Wafai & Koul, 1982), *Allium textile* (Alliaceae) (Khaleel & Mitchell, 1982) and also *Bellevalia edirnensis* (Hyacinthaceae) (Dane, 2006). On the other hand, 1-8 micronuclei were seen in the tetrads of the artificial F_1 orchid hybrids. Darlington (1989) clarified that meiotic irregularities in both primitive and advanced groups of plants were resulted from the genetic defects and hybridization. In this study, a few meiotic irregularities were seen in *L. aestivum.* This could be caused by environmental factors as well as genetic instability. Lagging chromosomes did not reach the poles as a result of the absence of spindle fibers or irregular formation. This has a role in the micronuclei formation. In another case, on the other hand, PMCs were mitotically divided without reducing the chromosome number, chromosomes move towards the poles by

seperating longitudinally and form diads in which chromosome number has not been reduced. Chromosome distribution is generally irregular between these dyads. Size of the nuclei is also different in dyads with unequal chromosome number. Some irregular chromosome-couplings leads to the delay in their movements towards the poles and causes them to be left in the middle of the cell. Some of these disappear and finally microspores with no equal size of nuclei are formed. However, some of them do not disappear and form micronuclei (Silva-Stort, 1984).

In *L. aestivum* like in the other Amaryllidaceae members, linear and T-shaped tetrads were rarely seen besides isobilateral, deccusate tetrads (Davis, 1966). On the other hand, in *Aristolochia elegans* L. (Aristolochiaceae) and *Sparganium erectum* L. (Sparganiaceae), 5 different types of tetrad were seen (Ünal, 2004). 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 *L. aestivum* as in *Sternbergia lutea* (L.) Ker-Gawler ex Sprengel (Dane, 1998) and *Bellevalia edirnensis* (Dane, 1999). 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 monocotyledones. 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 monocotyledones, 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 our observations related to pollen mitosis of *L. aestivum.* As in the other Amaryllidaceae members (Davis, 1966), mature pollen grains are two celled when shed from the anther in *L. aestivum.* Generative cell nucleus is long and lens-shaped; on the other hand, the vegetative cell nucleus is lobed. Mature pollen grains two celled in *Sternbergia lutea* (Amaryllidaceae) (Dane, 1998), *Bellevalia edirnensis* (Hyacinthaceae) (Dane, 1999), *Tulipa clusiana* (Wafai & Koul, 1982) and *Gagea stipitata* (Koul et al., 1976), three celled in *Allium textile* (Alliaceae) (Khaleel & Mitchell, 1982) two or three celled in some of the species of the Araceae family (Davis, 1966) when shed. Mature pollen grains of *L. aestivum* are monocolpate as in *Galanthus ikariae* Baker and *G. rizehensis* Stern (Şahin, 1997). Pollens of *L. aestivum* and *G. rizehensis* are prolate; however, pollens of *G. ikariae* are subprolate.

Meiotic irregularities in sporogeneous cells cause spores to lose their viability. These spores cannot 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 pollen loose 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 *L. aestivum* this ratio was determined as 1.77 %. 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). In *L. aestivum*, the tapetum is normally developped. The cause of pollen sterility is meiotic irregularities.

Mature pollen grains of *L. aestivum* was germínated in *in vitro* medium. Germination percentage of mature pollen grains was identified as 73 %. During the formation of pollen tube, some differences in the behavior of the tube nucleus were observed. In two celled pollen grains, the cytoplasm filled 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, vegetative nucleus usually firstly moves from the pollen grain. Even though This was observed in many angiosperms, it was seen that generative nucleus has moved first in some pollen tubes while vegetative nucleus moves first in some pollen tubes of *Triticum aestivum* L. (Chandra and Bhatnagar, 1974) and *Vicia* species (Dane and Meriç, 1999). 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. The differences in nucleus behavior observed in pollen tube cells were also identified in *L. aestivum*. During *in vitro* pollen germination of *L. aestivum,* some abnormal tubes were seen besides normal tubes as in *Vicia galileae* Plitm. & Zoh. (Fabaceae) (Dane and Meriç, 1999). It was observed that some pollen tubes were developed less and some of the had swollen tube tips. This has been caused by the abnormalities in the nuclei of some microspores (Ünal, 2004).

5. Conclusion

Some cytological and embryological characteristics of *L. aestivum* are studied for the first time. Some irregularities were determined in the development of the male gametophyte but these irregularities do not affect fertility of plant. And also *Leucojum*'s tapetum is different from *Galanthus* the sister genus. The data that we gained from this study may contribute to embryological characteristics which were used in taxonomy of Amaryllidaceae (Meerow et al., 1999) family.

6. Acknowledgement

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7. 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.)*. *American Journal of Botany*, Vol.66(3), pp.253-265, ISSN 0002-9122

Bhojwani, S.S. & Soh, W.Y. (2001). *Current Trends in the Embryology of Angiosperms,* Kluwer Academic Publishers, ISBN 0-7923-6888-6, Dordrecht, The Netherlands.

Brown, R.C. & Lemmon, B.E. (1992). Pollen development in Orchids 4. Cytoskeleton and Ultrastructure of the unequal pollen mitosis in *Phalaenopsis*. *Protoplasma*, Vol.167, pp.183-192, ISSN 0033-183X

Brown, R.C. & Lemmon, B.E. (1994). Pollen mitosis in the slipper orchid *Cypripedium fasciculatum*. *Sexual Plant Reproduction*, Vol.7, pp.87-94, ISSN 0934-0882

Chandra, S. & Bhatnagar, P. (1974). Reproductive Biology of *Triticum*. II. Pollen germination, pollen tube growth and its entry into the ovule. *Phytomorphology*, Vol.24(3&4), pp.211-217, ISSN 0031-9449

Cheung, A.Y. (1996). Pollen-pistil interactions during pollen tube growth, *Trends in Plant Science*, Vol.1, pp. 45-51, ISSN 1360-1385

Cresti, M., Blackmore, S. & Van Went, J.L. (1992). Atlas of Sexual Reproduction in Flowering Plants, Springer-Verlag, ISBN 3-540-54904-9, New York.

Dane, F. (1998). Mitotic divisions in root tip cells and pollen grains of *Sternbergia lutea* (L.) Ker-Gawl. Ex Sprengel (Amaryllidaceae). *Journal of Marmara for Pure and Applied Sciences*, Vol.14, pp.1-10. ISSN 1303-7412

Dane, F. (1999). Hekzaploid (2n=24) *Bellevalia edirnensis* Özhatay & Mathew'in polen mitozu ve polen morfolojisinin incelenmesi. *Turkish Journal of Biology*, Vol.23, pp.357-368, ISSN 1300-0152

Dane, F. (2006). Cytological and Embryological Studies of *Bellevalia edirnensis* Özhatay & Mathew (Hyacinthaceae). *Acta Botanica Hungarica*, September, Vol.57(3), pp.339-354, ISSN 0236-5383.

Dane, F. & Meriç, Ç. (1999). Reproductive biology of *Vicia* L. II. Cytological and cytoembryological studies on development of anther wall, microsporogenesis, pollen mitosis and male gametophyte in *V. galileae* Plitm. & Zoh. *Turkish Journal of Biology*, Vol.23, pp.269-281, ISSN 1300-0152

Darlington, C.D. (1989). Recent advances in cytology. (Genes, cells and organisms) Taylor & Francis ISBN 9780824013769, Mishawaka, USA

Davis, L.G., 1966, Systematic Embryology of the Angiosperms, Wiley, ISBN 101-224-154, New York, USA

Davis, P.H., 1984, Flora of Turkey and the East Aegean Islands. Edinburgh University Press, ISBN 0-7486-3777-X Edinburgh, United Kingdom

Deshpande, P.K., Bhuskute, S.M., Makde, K.H. (1986). Microsporogenesis and male gametophyte in some Cucurbitaceae. *Phytomorphology*, Vol.36(1,2), pp.145-150, ISSN 0031-9449

Ekici, N. & Dane, F. (2004). Polarity during sporogenesis and gametogenesis in plants. *Biologia, Bratislava* Vol.59(6), pp.687-696, ISSN 0006-3088

Furness, C.A., Rudall, P.J. (1999). Microsporogenesis in monocotyledons. *Annals of Botany*, Vol.84, pp.475-499, ISSN 0305-7364

Furness, C.A., Rudall, P.J. & Sampson, F.B. (2002). Evolution of microsporogenesis in angiosperms. *International Journal of Plant Sciences,* Vol.163, pp. 235-260, ISSN 1058-5893

Heinrich, M. (2004). Snowdrops: the heralds of spring and a modern drug for Alzheimer's disease. *The Pharmaceutical Journal,* Vol.273, pp.905-906, ISSN 0031-6873

Horner, Jr.H.T. (1977). A comparative light- and electron-microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile sunflower (*Helianthus annuus*). *American Journal of Botany*, Vol.64(6), pp.745-759, ISSN 0002-9122

Horner, Jr.H.T. & Lernsten, N.R. (1971). Microsporogenesis in *Citrus limon* (Rutaceae). *American Journal of Botany*, Vol.58(1), pp.72-79, ISSN 0002-9122

Jensen, W.A. (1962). Botanical Histochemistry. W. H. Freeman and Company, ISBN 3-87429-257-6, San Francisco, USA.

Johansen, D. A., 1940, Plant Microtechnique. Mc Graw-Hill Book Company, ISBN 0-07-032540-5, New York, USA

Khaleel, T.F. & Mitchell, B.B. (1982). Cytoembryology of *Allium textile* Nels. and Macbr. *American Journal of Botany*, Vol.69(6), pp.950-956, ISSN 0002-9122

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*, Vol.26(3), pp.255-263, ISSN 0031-9449

Meerow, A.W., Fay, M.F., Guy, C.L., Li, Q., Zaman, F.Q., Chase, M.W. (1999). Systematics of Amaryllidaceae based on cladistic analysis of plastid rbc*L* and trn*L-F* sequence data. *American Journal Of Botany,* Vol.86(9), pp.1325–1345, ISSN 0002-9122

Nadot, S.L., Forchoni, A. Penet, L., Sannier, J. & Ressayre, A. (2006). Links between early pollen development and aperture pattern in monocots. *Protoplasma*, Vol.228, pp.55-64.

Pankow, H. (1958). Über den pollenkitt bei *Galanthus nivalis*. *Flora* Vol.146, pp.240-253, ISSN 0367-2530

Park, S.K. Howden, R. & Twell D. (1998). The *Arabidopsis thaliana* gametophytic mutation gemini pollen 1 disrupts microspore polarity, division asymmetry and pollen cell fate, *Development*, Vol.125, pp. 3789-3799, ISSN 1011-6370

Sahin, N.F. (1997). An investigation on pollen morphology of *Galanthus ikariae* Baker and *Galanthus rizehensis* Stern (Amaryllidaceae). *Turkish Journal of Botany*, Vol.21, pp.305-307, ISSN 1300-008X

Sajo, M.G., Furness, C.A. & Rudall, P.J. (2009). Microsporogenesis is simultaneous in the early-divergent grass *Streptochaeta,* but successive in the closest grass relative, *Ecdeiocolea. Grana*, Vol.48, pp.27-37, ISSN 0017-3134

Sharma, M. & Vij, S.P. (1987). Embryological studies in Orchidaceae VI: *Habenaria willd*. *Phytomorphology,* Vol.37(4), pp.327-335, ISSN 0031-9449

Shull, J.K. & Menzel, M.Y. (1977). A study of reliability of synchrony ın the development of pollen mother cells of *Lilium longiflorum* at the first meiotic prophase. *American Journal of Botany*, Vol.64(6), pp.670-679, ISSN 0002-9122

Silva-Stort, M.N. (1984). Sterility barriers of some artificial F1 Orchid hybrids. I. Microsporogenesis and pollen germination. *American Journal of Botany*, Vol.71(3), pp.309-318, ISSN 0002-9122.

Ünal, M. 1986. A comparative cytological study on compatible and incompatible polen tubes of *Petunia hybrida, Journal of Istanbul University, Faculty of Science*, Series B. Vol.51, pp.1-12, ISSN 0367-7753

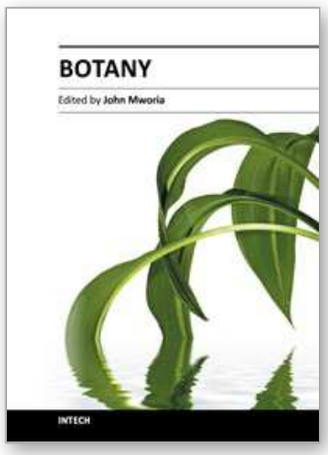
Ünal, M. (2004). Plant (Angiosperm) Embryology. ISBN 975-400-040-9, İstanbul, TURKEY.

Wafai, B.A. & Koul, A.K., (1982). Analysis of breeding systems in *Tulipa*. II. sporogenesis, gametogenesis and embryogeny in tetraploid *T. clusiana*. *Phytomorphology,* Vol.32:(4), pp.289-301, ISSN 0031-9449

Watson, L., Dallwitz, M.J., 2005, The Families of Flowering Plants. http://delta-intkey.com/angio/www/amaryllidaceae.htm.

Zenkteler, M. (1962). Microsporogenesis and tapetal development in normal and male-sterile carrots (*Daucus carota*). *American Journal of Botany*, Vol.49(4), pp.341-348, ISSN 0002-9122

http://www.amaryllidaceae.org/Leucojum/



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This book is devoted to botany and covers topical issues in this diverse area of study. The contributions are designed for researchers, graduate students and professionals. The book also presents reviews of current issues in plant-environment interactions making it useful to environmental scientists as well. The book is organized in three sections. The first section includes contributions on responses to flood stress, tolerance to drought and desiccation, phytotoxicity to Chromium and Lead; the second has aspects of economic botany including a review of Smut disease in sugarcane and properties of plant extract used Tassaboount date juice; the last covers topical issues on morphogenesis and genetics on cotton fiber special cell, secretory glands Asphodelus aestivus flower ,pollen tube growth in Leucojum aestivum , morphological studies of Ardisia crenata complex, and hybrid lethality in the Genus Nicotiana.

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