Journal of Applied and Natural Science 8 (1) : 144 - 149 (2016)

JANS

Pollen morphology in two *Urgineoid* species of *Drimia* (Baker) Jessop

P. Prathima[1], M. N. Shiva Kameshwari[2]* and R. Nijagunaiah[3]

[1]Research and Development Centre, Bharathiar University, Coimbatore - 641 046, INDIA
[2&3] *Department of Botany, Jnana Bharathi Campus, Bangalore University, 560056. INDIA
*Corresponding author: E-mail. mn.shivakameshwari@gmail.com

Received: May 15, 2015; Revised received: October 30, 2015; Accepted: February 4, 2016

Abstract: Pollen grains of eight accessions of *Urginea indica* (Roxb) Kunth. and two accessions of *Urgineawightii* (Wight) Lakshmin. were examined by Light microscopy and Scanning electron microscopy. Detailed pollen morphological characters are given for these accessions. Our investigation revealed several variations in pollen size, exine ornamentation among the accessions of *U. indica* (Roxb) Kunth. and *U. wightii* (Wight) Lakshmin. The study confirmed that the exine sculpting provides valuable characters for separating the species of *Urginea.* Reticulate ornamentation of the exine was observed in the accessions of *U. indica* complex and perforated exine ornamentation in accessions of *U. wightii* complex. Minor variations with regard to measurements of dimensions in size, shape and ornamentation were also observed in accessions of *U. indica* and *U. wightii* . Pollen fertility analysis revealed highest percentage fertility in Sitampoondi accession of *U. indica* complex and Gulbargha accession of *U. wightii* complex but in most of the accessions seed setting is not observed in natural conditions. Pollen germination studies revealed highest germination rate in Brew Baker's and Kwack medium supplemented with 25% sucrose concentration for *Urginea* pollen.

Keywords: Accessions, Hyacinthaceae, Pollen fertility, Scanning electron microscope, *Urginea indica, Urginea wightii*

INTRODUCTION

Urginea Steinhill.Hyacinthaceae is one of the interesting polytypic genus with about 100 species occurring in India, Africa, Mediterranean regions and in the plains of South India (Airy Shaw, 1965). The genus *Urginea* is a bulbous geophyte with species showing phenotypic plasticity. The genus was for the first time investigated cytologically by Raghavan (1935). Shiva Kameshwari and Muniyamma (2004) have made extensive studies and have demonstrated the presence of natural polyploidy in the genus. *Urginea* is known for a wide range of morphological and cytological variations. This must have misled the previous workers to make erroneous identifications and arrive at wrong conclusions. It is well known that pollen features have a great taxonomic value, and have been used in the classification of different genera (Troia *et al*.,2012; Ceter *et al*.2013) and also closely related Liliaceae taxa (Teksen *et al*. 2010;Masoumi 2012). A comparative study of the pollen morphology employing electron microscopy has been made by Kosenko (1999). The pollen morphology of *Nomocharis* with *Lilium* of Liliaceae were investigated under both LM and SEM to elucidate the systematics of the genera by Liang and Zhang (1985). Similar studies were carried out in *Lilium* (Liliaceae) by Seher Guven *et al*. (2014). Palynological studies investigated in Liliaceae members indicated that palynological characters such as sulcus, muri and lumina are taxonomically useful Kuprianova (1983); Kosenko (1999); Shiva Kameshwari (2011). Pollen grains although minute show many characters of taxonomic and phylogenetic importance. The present study aims at detailed pollen morphological characters for eight accessions of *U. indica* (Roxb) Kunth. and two accessions of *U. wightii* (Wight) Lakshmin. The investigation also evaluates the taxonomical relationship between the species and accessions of *Urginea* from a pollen morphological perspective and test the viability of pollen grains and *in vitro* pollen germination of few accessions.

MATERIALS AND METHODS

In the present investigation eight accessions of *U. indica* and two accessions of *U. wightii* were collected, identified, and maintained in the germplasm at Department of Botany, Bangalore University, Bangalore.

Phenology: Phenological events (time of leaf fall, renewal, flowering and fruiting period) were recorded. Morphometrical studies of flowering phenology, counting the number of flowers, length of the inflorescence, perianth, androecium and gynoecium of the accessions periodically for about 4 years throughout the flowering period (March to May), blooming time of the flowers were observed and recorded.

Light microscopic studies: Pollen of eight accessions of *U. indica* and two accessions of *U. wightii* were collected from various localities and were used for LM studies. For LM investigations pollen slides were

ISSN : 0974-9411 (Print), 2231-5209 (Online)

prepared by mounting pollen grains from freshly dehisced anther suspended in 50% glycerin and mounted on a slide. Pollen size was measured with an ocular micrometer under light microscope as described by Moore and Webb (1978).

Scanning electron microscopic studies: Pollen grains were transferred to aluminum stubs and coated with gold and examined under a JOEL JSM 5600 Scanning electron microscope. Pollen morphometric measurements were made using Image J software. The figures reported are the average of 20 measurements of the pollen grains per accessions

Pollen fertility: Fresh pollen of 10 accessions of *U. indica* and 3 accessions of *U. wightii* were collected at anthesis. TTC (2,3,5-triphenyl tetrazolium chloride) stain test was used for this purpose after the method of Hauser and Morrison (1964). The pollen fertility tests were tested individually for each accession for both the species. A few drops of 0.5% TTC were dropped by pipettes on microscope slides and pollen were dusted with a slim brush (each brush used only one accession type) covered with a cover slip and 3 different areas of each cover slip were counted for TTC tests. The fertile pollen grains stained with TTC dyed majenta color were considered as viable. Those pollen grains which do not show this effect were classified as unviable or sterile. Pollen fertility was determined as per the formula.

Percentage Pollen Fertility = Number of Fertile pollen grain stained / Total number of Pollen grains X 100

***In vitro* pollen germination:** Brew Baker's and Kwack (1963) culture medium with different sucrose concentrations (5%, 15% and25%) were used for the pollen germination studies in accession number 835 (Ramanagaram) of *U.indica* complex.

RESULTS AND DISCUSSION

Pollen morphological attributes can be used both to distinguish related taxa or to integrate them into a common group (Sevil Pehlivan 2003). Pollen exine sculpturing is considered to be as evolutionary reversible character but the internal wall structures are thought to be more stable and are important in assessing affinities with other families in the order of Liliales (Walker and Doyle 1975).

Monosulcate pollen grains are a primitive trait in seed plants and occur widely among the monocotyledons (Furness and Rudall 2001). According to LM and SEM investigations, the pollen grains of various accessions confirmed that all accessions of the genus *U. indica* and *U. wightii* were found to be monosulcate and ellipsoidal (Figs. 1 and 2). The color of the pollen grains of the examined accessions belonging to both the species of *Urginea* were yellow .The predominance of monosulcate aperture is emphasized by Harley and Zavada (2000). Similar results were reported in genus *Lilium* by Kosenko (1999), Pupuleku *et al.* (2010) and Muratovic *et al.* (2010).

The two *Urginea* species were homogenous in possessing a single longitudinal aperture (monosulcate). Finer differences and variations do exist in the reticulation of the exine between the species and accessions as observed in *U. indica* complex and *U. wightii* complex. Variation in the aperture examined in the pollen grains of accessions in both the species can be used to delimit the species. The main features of the investigated pollen are summarized in Tables 1 - 4. Large boat shaped, ellipsoidal monosulcate pollen grains with reticulate and perforated exine ornamentation are the most common type of pollen found in *U. indica* and *U. wightii*. Palynological measurements are summarized in Table 1 and Table 2. During the present study remarkable degrees of variations in the measurements of dimensions were observed between the accessions of *Urginea*. Among the examined accessions of *U. indica* complex, biggest pollen size was found in accession number 842 (Kerala) and smallest in accession number 845 (Coimbatore). In accessions of *U. wightii* complex biggest pollen size was observed in accession number 825 (Gulbarga).

Studies in pollen viability and morphology play a role in genetic breeding programmes. Determination of pollen fertility or viability can be assessed by the use of direct methods such as the inducement of *in vitro* germination (Acar and Kakani 2010; Alcaraz *et al.,* 2011; Sorkheh *et al.* 2011) and *in vivo* germination

***Urginea indica* (Roxb) Kunth.**
Accession Number (835)

***Urginea wightii* (Wight) Lakshmin.**
Accession Number (839)

Table 1. Pollen morphological parameters in accessions of *U. indica* (Roxb.)Kunth

Sl. no	Name of the Accession and number	Long axis (µm)	Short axis (µm)	Ornamentation	Flowering Time	Chromosome number	Ploidy
1	Kerala (842)	84.99	40.22	Reticulate	Evening	50	Pentaploid
2	Ramanagaram (835)	74.49	28.80	Reticulate	Evening	20	Diploid
3	Trichy (844)	77.96	31.55	Reticulate	Evening	-	-
4	Biligirirangana hills (836)	84.28	33.14	Reticulate	Evening	36	Aneuploid
5	Gopalaswamy betta (807)	77.35	29.96	Reticulate	Afternoon	40	Tetraploid
6	Coimbatore (845)	73.10	31.47	Reticulate	Evening	-	-
7	Ranganthittu (804)	83.56	26.13	Reticulate	Night	34	Aneuploid
8	Shimoga (802)	78.23	32.79	Reticulate	Morning	20	Diploid

Table 2. Pollen morphological parameters in accessions of *U. wightii* (Wight) Lakshmin.

S. N.	Name of the Accession and number	Long axis (µm)	Short axis (µm)	Ornamentation	Flowering time	Chromosome number	Ploidy
1	Gubbi (848)	62.50	24.02	Perforated	Afternoon	20 and40	Diploid and Tetraploid
2	Gulbargha (825)	78.75	31.67	Perforated	Morning	36	Aneuploid

Table 3. Percentage fertility in *U. indica* accessions.

S.N.	Name of the Accession and number	% Fertility	Chromosome number	Ploidy
1	Banganwadi (815)	25.92%	40	Tetraploid
2	Karighatta (826)	77.21%	34	Aneuploid
3	Sitampoondi (846)	95.5%	20	Diploid
4	Channamallipura (809)	6.52%		
5	Coimbatore (845)	93.22%		
6	Gopalaswamybetta (807)	76.19%	40	Tetraploid
7	Ramanagaram (835)	6.45%		
8	Ranganthittu 804	7.936%	34	Aneuploid
9	Magadi (834)	91.66%	20	Diploid
10	Shimoga (802)	5.376%	20	Diploid

(Fakhim *et al.* 2011) or other, indirect methods based on cytological parameters, such as pollen staining (Beyhan and Serdar 2008; Abdelgadir *et al.* 2012). Pollen fertility was assessed through histochemical analysis using 0.5%. 2,3,5-triphenyltetrazolium chloride (TTC) solution. Pollen Fertility analysis indicated highest in accession number 846 (Sitampoondi) and lowest in accession number 802 (Shimoga) of *U. indica* complex and highest in accession number 825 (Gulbarga) and lowest in accession number 848 (Gubbi) of *U. wightii* complex (Table 3 and 4). Seed setting was observed in these three accessions which indicated highest fertility rate (accession number 846, 825 and 839) but seed setting is not observed in majority of the accessions in natural conditions.

In vitro analysis of pollen germination studied in accession number 835 (Ramanagaram) presented the highest germination rate in Brew Baker's and Kwack (1963) medium with 25% sucrose concentration (Fig 3). During pollen development, T.S. of microsporangium revealed normal and shrunken pollen grains (Fig. 4) with vegetative and generative nucleus. The exine structure of pollen grains comprised tectum and collumellae. Variations in exine thickness is an important palynological trait examined in different accessions of *U. indica* and *U. wightii*. Ornamentation characteristics of the pollen grains of the investigated accessions as observed in SEM micrographs have been used for their diagnostic features and accordingly reticulate ornamentation were observed in *U. indica* and perforated ornamentation in *U. wightii.* According to Neetin Desai et.al (2012) Indian *Drimia (Urginea)* species cannot be distinguished on the basis of their pollen morphology as they have more similarity than differences. In the present investigation reticulate ornamentation was observed in *U. indica* complex and perforated ornamentation in *U. wightii* complex.

For resolving taxonomic misunderstandings morphological characters have the most important role in plant systematics, palynological,and anatomical characters can be used to support the morphological characters. Ploidy plays an important role in delimiting the taxa. Genome size is the predictor of pollen size. According to Charles Knight et al., (2010), pollen is not a good candidate to relate with the genome size but

Table 4. Percentage fertility in *Urginea wightii* accessions

S. N.	Name of the Accession and number	% Fertility	Chromosome number	Ploidy
1	Gulbarga (825)	92.13%	36	Aneuploid
2	Yediyur (839)	91.83%	20	Diploid
3	Gubbi (848)	52.63%	20 and 40	Diploid and Tetraploid

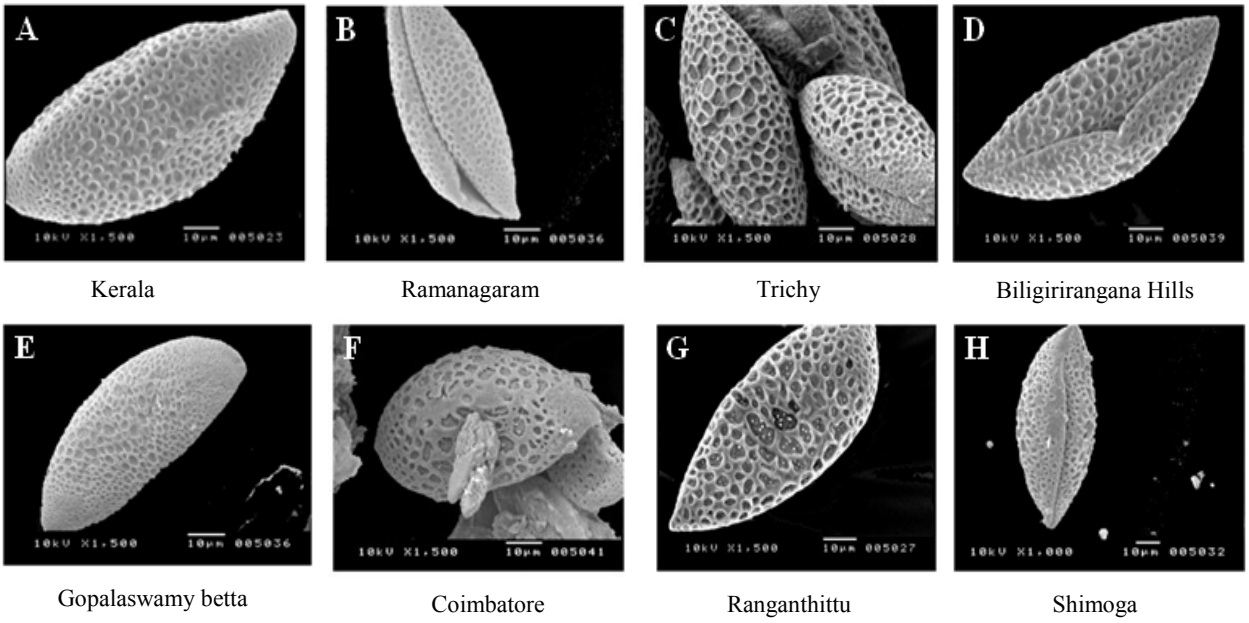

Fig 1: ***A – H.*** *SEM photographs of pollen grains of Urginea indica accessions showing reticulate sculpturing. A - Proximal view of accession number 842 (Kerala).* ***B*** *– Distal view of accession number 835 (Ramanagaram).* ***C*** *– Proximal view of accession number 844 (Trichy).* ***D*** *– Distal view of accession number 836 (Biligirirangana hills).* ***E*** *– Proximal view of accession number 807 (Gopalaswamy betta).* ***F*** *– Proximal view of accession number 845 (Coimbatore).* ***G*** *– Proximal view of accession number 804 (Ranganthittu).* ***H*** *– Distal view of accession number 802 (Shimoga).*

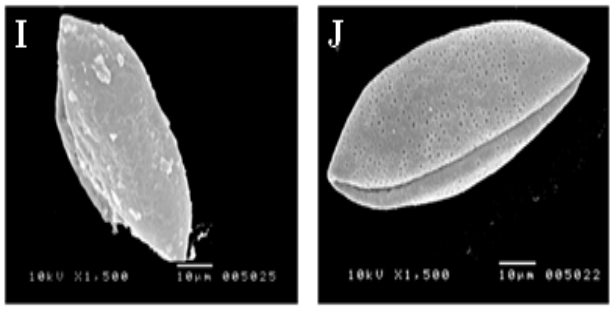

Fig 2: I – J. *SEM photographs of pollen grains of Urginea wightii accessions showing perforated ornamentation. I – Proximal view of accession number 848 (Gubbi). J – Distal view of accession number 825 (Gulbargha).*

in our present investigation, there is considerable variation in pollen size with respect to ploidy. accession number 842 being pentaploid showing biggest pollen grain in the *U. indica* complex whereas in *U. wightii* complex, accession number 825 an aneuploid shows biggest pollen grains indicating that ploidy plays an important role in delimiting the taxa.

Conclusion

Our investigation confirms that pollen characters in *Urginea* were of taxonomic significance. Differences were encountered in pollen shape, size, ornamentation, sulcus membrane ornamentation, apex of sulcus, muri and lumina. The exine ornamentation observed in *U. indica* complex and *U. wightii* complex reveals that it is one of the valuable characters for separating the two species. Reticulate ornamentataion was observed in *U indica* complex and perforated ornamentation in *U. wightii* complex. There is also intraspecific variation observed between the accessions of both the complexes which indicates the heterogeneity of the species. The study of the pollens of *Urginea* highlights that features like size related to ploidy will be useful in assessing and evaluating the taxonomic relationships between the species and the accessions. Pollen morphological features provide additional valuable taxonomic information in separating the accessions of *U. indica* (Roxb)Kunth. and *U. wightii* (Wight) Lakshmin. and may be regarded as more dependable and useful criteria in studies on comparative morphology and taxomomy.

REFERENCES

Abdelgadir, H.A., Johnson, S.D. and Van Staden, J. (2012). Pollen viability, pollen germination and pollen tube growth in the biofuel seed crop *Jatropha curcas* (Euphorbiaceae). *South African Journal of Botany,* 79: 132-139.

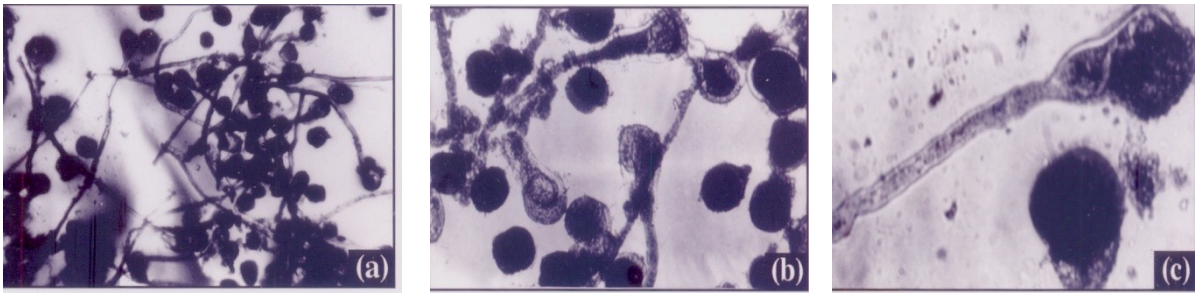

Fig 3. ***(a)*** *–* ***(b)*** *Pollen germination.* ***(c)*** *Pollen tube formation of accession number 835 (Ramanagaram).*

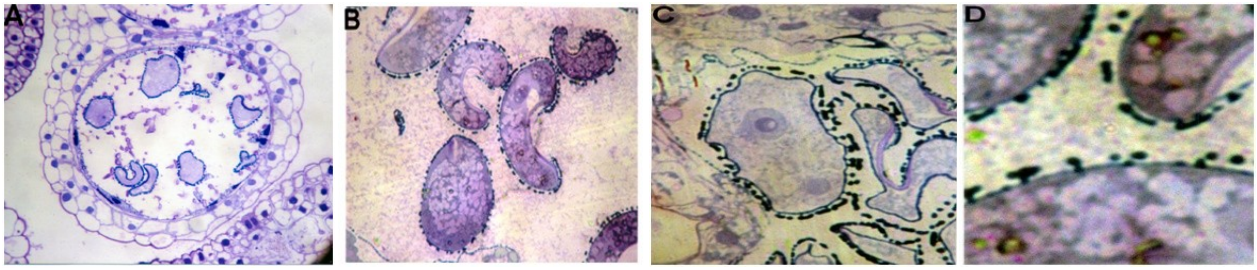

Fig. 4. *Pollen development in Urginea indica accession number 835 (Ramanagaram).* ***A*** *– T.S. of Microsporangium.* ***B*** *- Development of Pollen grain.* ***C*** *– Pollen grain showing vegetative and generative nucleus.* ***D*** *- Pollen grains with tectum and collumellae.*

Alcaraz, M.L.,;Montserrat, M, and. Hormaza, J.I.(2011). *In vitro* pollen germination in avocado (*Persea americana* Mill.): Optimization of the method and effect of temperature. *Scientia Horticulturae*, Amsterdam, 130: 152-156.

Airy- Shaw, H.K. (1965). Diagnoses of new families, new names etc. for the seventh edition of Willis's Dictionary, Kew, Bulletin, 18: 249 – 273.

Beyhan, N. and Serdar, U. (2008). Assessment of pollen viability and germinability in some European chestnut genotypes (*Castanea sativa* L.). *Hort Science* 35: 171-178.

Brewbaker J. L.and Kwack B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany* 50: 859-865.

Carol.A.Furness and Paula. J. Rudall (2001). Pollen and other characters in monocot systematics.Grana, 40: 1 – 2, 17 – 25.

Ceter T., Ekici M., Pinar NM. and Ozbek F. (2013). Pollen morphology of *Astragalus* L. section Hololeuce Bunge (Fabaceae) in Turkey. *Acta Botanica Gallica* 160: 43 – 52.

Charles A.Knight., Rachel B. Clancy, Lars Gotzenberger, Leighton Dann, and Jeremy M.Beaulieu.(2010). On the Relationship between Pollen size and Genome size. Hindawi Publishing Corporation *Journal of Botany* 7 pages.

Fakhim, R.S.H.; Hajilou, J. and Zaare, N.F. (2011). Pollen germination and pistil performance in several Iranian peach cultivars. *International Journal Agri Science* 1: 71-77.

Harley, M. M., and M. S. Zavada. (2000). Pollen of the monocotyledons: selecting characters for cladistic analysis. 191–210.

Hauser, E. J. P., and Morrison, J. H. (1964). The cytochemical reduction of nitroblue tetrazolium as an index of pollen viability. *American Journal of Botany,* 51: 748-752.

Izzet Acar , Vijaya Gopal Kakani (2010). The effects of temperature on *in vitro* pollen germination and pollen tube growth *of Pistacia* spp. *Scientia Horticulturae* 125 : 569–572.

Kosenko, VN. (1999). Contributions to the pollen morphology and taxonomy of the Liliaceae. *Grana* 38: 20 – 30.

Liang, S. Y. and Zhang, W. X. (1985). Pollen morphology of the genus *Nomocharis* and its delimitation with *Lilium*. *Acta Phytotax*. 23: 405 - 417.

Mehtap Tekşen, Zeki Aytac, Nur Munevver Pinar (2010). Pollen morphology of the genus *Fritillaria* L. (Liliaceae) inb Turkey *Turk J Bot* 34: 397- 416.

Moore, P.D.,and Webb, J.A., (1978). An illustrated Guide to Pollen Analysis, Hodder and Stoughton, London

Muratovic E., Bogunic F., Soljan, D. Martin,J.,Vales,J.,Sonja Siljak Yakovlev (2010) Stomata and pollen grain characterstics of two endemic lilies:*Lilium bosniacum* and *L.carniolicum* (Liliaceae) *Phytologia Balcanica* 16: 285 – 292.

Neetin Desai .,Hemant Kawalkar and Ghansham Dixit (2012)Biosystematics and evolutionary studies in Indian *Drimia* species *Journal of Systematics and Evolution* 50 (6): 512–518 .

Pupuleku B., Kapidani G., Kallajxhiu N., Naqellari P. and Turku S. (2010)_. Palynological Study of Pollen grains of Albania's Endemic plant *Festucopsis serpentini* (C.E.Hubbard) Melderis and *Lilium martagon* L. in three different habitats. *Natura montenegrina* 9: 431-440.

Sayed-Mohammad Masoumi (2012) Exine Ultrastructure of *Fritillaria* (Liliaceae Juss) *J. Basic. Appl. Sci. Res.,* 2 (2)1101-1109.

Seher Guven , Seda Okur, Mine Sezen Dem˙irel , Kamil Coskuncelebi , Serdar Makbul and Osman Beyazoglu (2014) Pollen morphology and anatomical features of *Lilium* (Liliaceae) taxa from *Turkey Biologia* 69/9: 1122—1133.

Sevil Pehlivan, Hulya Özler (2003) Pollen morphology of some species of *Muscari* Miller (Liliaceae-Hyacinthaceae) from Turkey *Flora* 198, 200–210.

Shiva Kameshwari M.N.,(2011). Pollen morphology in some members of Liliaceae. *International Journal of Engineering Science and Technology*. 3: 3825 – 3830.

Shiva Kameshwari, M. N. and Muniyamma, M. (2004). Karyomorphological Studies in *Urginea indica* Kunth. (Liliaceae) of Karnataka in India *Beitrage Biologie der Pflanzen*, 73: 377-394.

Sorkheh, K.; Shiran, B.; Rouhi, V.; Khodambashi, M.; Wolukau, J.N. and Ercisli, S. 2011. Response of *in vitro* pollen germination and pollen tube growth of almond (*Prunus dulcis* Mill.) to temperature, polyamines and polyamine synthesis inhibitor. *Biochemical Systematics and Ecology* 39: 749-757.

Troia A., Raimondo F.M., Castellano G. and Spadaro V. 2012. Morphological, karyological and and taxonomic remarks on *Ferulago nodosa* (L.) Boiss. (Apiaceae). *Plant Biosyst*. 146: 330 – 337.

Walker, J.W. and Doyle, J.A. (1975) The bases of angiosperm phylogeny: palynology. *Annals of the Missouri Botanical Garden* 62, 664-723.