COMPARISON OF POLLEN MORPHOLOGICAL STRUCTURES OF SOME TAXA BELONGING TO ASPARAGUS L. AND FRITILLARIA L. (LILIACEAE) FROM TURKEY

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Key words: Asparagus, Fritillaria, Liliaceae, Pollen morphology

Abstract

Pollen grains of 20 taxa from two genera of the Liliaceae were examined and compared by LM (light microscope), SEM (scanning electron microscope) and pollens of four taxa were also examined with TEM (transmission electron microscope). Pollen grains shed as monads. They are monosulcate and ellipsoidal. *Fritillaria crassifolia* subsp. *crassifolia* Freyn & Smt. sometimes sheds the pollen as dyads. Exine is semitectate and the tectum is perforate. Columellae are simplicolumellate. Ectexine is thicker than endexine. Exine sculpture (ornamentation) is reticulate, reticulate-rugulate, rugulate and retipilate in *Asparagus* pollens and reticulate, suprareticulate, rugulate-reticulate and striate-reticulate in *Fritillaria* pollens. Sulcus extends from distal to proximal in some pollens of *Asparagus* and *Fritillaria*.

Introduction

The Liliaceae contains approximately 250 genera and 3500 species. There are approximately 400 Liliaceous species in Turkey and it is one of the richest families. According to recent studies, 49 taxa of *Fritillaria* and 13 taxa of *Asparagus* are available in Turkey. The endemism ratio is 36.53% in *Fritillaria* and 23% in *Asparagus*. These genera have economic and pharmaceutical importance (Davis et al. 1984, 1988, Seçmen et al. 1998, Güvenç 1996, Guner et al. 2000). No detailed palynological studies have been found concerning these genera except for the LM studies by Radulescu (1973) and Schulze (1980, 1982); by Gori (1982), El-Ghazali (1993), Kosenko (1991a, 1991b, 1992, 1999) and SEM and TEM studies. Among monocotyledones plants, the Liliaceae family is a difficult group due to taxonomical and systematic reasons. Some authors explained that the comparative study of pollen morphology of the whole Liliaceae by SEM and TEM hasn’t been studied in detail yet (Zavada 1983, Doyle and Hotton 1991, Kosenko 1999a). In order to obtain more morphological data to solve some of these problems, the pollen grains of 20 taxa were comparatively examined in detail for the first time.

Materials and Methods

The pollen materials were obtained from the Faculty of Pharmacy of Ankara University, the Faculty of Science of Hacettepe University and the Faculty of Arts and Science, Gazi University herbariums. For the light microscopy (LM) investigations, pollen grains of 20 taxa, taken from herbarium materials, were prepared according to the methods of Wodehouse (1935) and Erdtman (1960). Pollen dimensions of all species were measured in such amounts that the resulting data followed Gaussian curves. These measurements are shown in Table 1. For scanning electron microscopy (SEM) investigations, the pollen grains were put on stubs, sputter-coated with gold plate and examined under a Jeol JSM-840A scanning electron microscope. For transmission electron microscopy (TEM) studies, acetylated pollen grains were stained with 2 % OsO₄ and with uranyl acetate, dehydrated and embedded in epon-araldite according to the method described by Skvarla and Turner (1966). Ultrathin sections of the pollen grains were obtained with a glass knife in a...
Reichert Supernova microtome. Post staining was performed with lead citrate for 5 minutes (Reynold 1963) and the sections were examined under a Zeiss EM9. The pollen morphological terminologies by Walker (1974 a, b), Faegri and Iversen (1989) and Punt et al. (1994) were used.


Results and Discussion

General remarks to the genus: Asparagus

Pollen grains of 8 Asparagus species, 3 of which are endemic, are monads monosulcate, ellipsoidal. Exine is semitectate and the tectum is perforate. Exine sculpture is reticulate, reticulate-rugulate, rugulate or retipilate. Sulcus extends from distal to proximal in the examined seven taxa with the exception of A. coodei. Sulcus membrane is granulate, rugulate or psilate. Sulcus ends are rounded in all investigated taxa. Sulcus becomes narrow at the equator, widens at the poles. The number of the lumina in 1 µm² is 1-12, the diameter of lumina is approximately 0.10-0.55 µm, the number of perforation in 1 µm² is 2-6 at the sulcus side or at the lateral surface. The diameter of a perforation is approximately 0.08-0.18μm and the thickness of a murus is on average 0.09-0.25 μm. Ectexine is thicker than endexine in TEM micrographs in A. verticillatus and A. lycaonicus. Ectexine is thicker in A. verticillatus than in A. lycaonicus. Endexine is rather thin in both species. Columellae are simplicolumellate (Figs. 1-10, 23-36, 59, 60).

Fritillaria

Pollen grains of 12 endemic taxa were shed as dyads in F. crassifolia subsp. crassifolia (5 %) and the other species were monosulcate, and ellipsoidal. Exine was semitectate and the tectum was perforate. Exine sculpture was reticulate, suprareticulate, rugulate-reticulate or striate-reticulate.
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Sulcus extended from distal to proximal in *F. aurea*, *F. bithynica* and *F. sibthorpiiana*. Sulcus membrane is gemmate or granulate. The number of granule in 2 µm² on sulcus membrane was approximately 2-9, the number of gemmae in 2 µm² was approximately two in *F. elwesii*. Sulcus end were rounded or sharp. At the lateral surface or at the edge of the sulcus the number of lumina in 1 µm² was 1-4, the diameter of lumina was approximately 0.17-0.97 µm, the number of perforation in 1 µm² was 3-7. The diameter of a perforation was approximately 0.12-0.25 µm and the thickness of a murus was on an average 0.20-0.74 µm. In the ultrathin sections of *F. armena* and *F. whittalli*, ectexine was thicker than endexine. Ectexine was thicker in *F. whittalli* than in *F. armena*. Endexine was very thin (Figs. 11-22, 37-58, 61,62).

In this study, a comparative pollen morphological study of 20 entomogamous taxa, 15 of which are endemic, were done using LM, SEM. Four taxa belonging to Asparagus and Fritillaria were also examined with TEM in addition to LM and SEM. The common characteristic of the pollen grains of the investigated taxa is that their pollen apertures are monad monosulcate. It was observed that only a small amount of dyad pollen was found in *F. crassifolia* subsp. *crassifolia*. Kosenko (1991a) found that *F. eduardii* had dyad and tetrad pollens. The predominance of monosulcate aperture in monocotyledons, as in Asparagales and Liliales, is emphasized by Harley and Zavada (2000). Liliaceae and overwhelming majority of Lilialean complex have monosulcate pollens. The evolution of distal sulcate pollen may have occurred in Mesozoic geological periods. Typically, monoaperturate *Asparagus* and *Fritillaria* pollens are shed as ellipsoidal monads. Monosulcate aperture may be strong palynological evidence for the common origin of monocotyledon and dicotyledons. Sulcate, colpate, colporate pollen apertures are the most common in biotically pollinated families and sulcate pollen has a much older pollen record than those of both porate and colpate pollen (Kuprianova 1969, 1979, Sporne 1972, Zavada 1983, Linder 2000).

During the present study, some remarkable differences in the measurement of dimensions were observed between taxa. Among the examined genera, the biggest pollen size was found in *F. sibthorpiiana* and *A. persicus* (Table 1). *F. armena* species studied by Kosenko (1991a), is discussed under *F. pinardii* Boiss in Turkish flora (Davis et al. 1988).

No data about sulcus extention to proximal and sulcus ends have been observed in any work other than those by Schulze (1980), Pehlivan and Ozler (2003) and Guler and Pehlivan (2006). Chanda et al. (1979) explained that extended sulcate type of aperture were not common and that were occasionally found in both dicotyledones and monocotyledones. The sulcus ends are rounded in all investigated *Asparagus* pollens and in *Fritillaria* pollen grains except for *F. aurea* and *F. bithynica* (Figs.1-20, 23-58). The longest length of the sulcus (Slg) was seen in *F. aurea*, the shortest was in *A. coodei*. In comparison to *Asparagus*, the widest sulcus was measured in *Fritillaria* pollens (Table 1). Some researchers have shown that the sulcus features may be a taxonomic characteristic in some families (Schulze 1980, 1982, Faegri and Iversen 1989, Kosenko 1991a, 1991b, 1992, 1999, Halbritter and Hesse 1993, Friis et al. 1997).

No other previous studies related to these genera provided data about the sulcus membrane characteristics except for the research on *Fritillaria* species by Kosenko (1991a, 1992, 1999). According to Kosenko (1991a, 1999) sulcus membrane surface in *Fritillaria* species were granular or plicate granular and sulcus membrane surface were good characteristics for classification of *Fritillaria* and its subspecies. It was found that sulcus membrane is gemmate in *F. elwesii* and granulate in other *Fritillaria* species pollens. Sulcus membrane of *Asparagus* pollen grains are granulate and psilate. The edges of the sulcus are irregular in investigated taxa (Table 1, Figs.1-21, 23-58).

Semitectate, tectum perforate exine and thin exine in the examined taxa are primitive characteristics in monocotyledones and are observed in the early stages of angiosperm evolution (Radulescu 1973, Schulze 1980, 1982, El-Ghazali 1993, Kosenko 1991a, 1991b, 1999, Furness
### Table 1. Pollen morphological parameters of *Asparagus* and *Fritillaria* taxa.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>A/μm</th>
<th>B/μm</th>
<th>A/B</th>
<th>Slg/μm</th>
<th>Slt/μm</th>
<th>Exine/μm</th>
<th>Intine</th>
<th>Orn</th>
<th>SMO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. acmopetala</em></td>
<td></td>
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</tr>
<tr>
<td>sub sp. wendelboi (W)</td>
<td>50.37</td>
<td>±1.84</td>
<td>34.60</td>
<td>±2.12</td>
<td>1.45</td>
<td>45.15 ± 2.67</td>
<td>12.28 ± 3.14</td>
<td>1.93±0.52</td>
<td></td>
</tr>
<tr>
<td>subsp. wendelboi (E)</td>
<td>54.95</td>
<td>±4.53</td>
<td>40.15</td>
<td>±3.21</td>
<td>1.36</td>
<td>48.00 ± 3.67</td>
<td>14.80 ± 4.72</td>
<td>2.08±0.33</td>
<td></td>
</tr>
<tr>
<td><em>F. whiteii</em> (W)</td>
<td>45.07</td>
<td>±2.81</td>
<td>34.71</td>
<td>±3.08</td>
<td>1.29</td>
<td>40.32 ± 3.28</td>
<td>21.34 ± 3.44</td>
<td>1.62±0.31</td>
<td></td>
</tr>
<tr>
<td><em>F. whiteii</em> (E)</td>
<td>54.30</td>
<td>±2.45</td>
<td>42.84</td>
<td>±2.03</td>
<td>1.26</td>
<td>49.82 ± 4.53</td>
<td>22.01 ± 4.89</td>
<td>2.00±0.20</td>
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<tr>
<td><em>F. crassifolia</em></td>
<td></td>
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<tr>
<td>subsp. crassifolia (W)</td>
<td>44.73</td>
<td>±2.5</td>
<td>33.24</td>
<td>±1.79</td>
<td>1.34</td>
<td>39.16 ± 3.22</td>
<td>13.06 ± 2.17</td>
<td>1.90±0.26</td>
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</tr>
<tr>
<td>subsp. crassifolia (E)</td>
<td>55.32</td>
<td>±3.10</td>
<td>37.04</td>
<td>±3.80</td>
<td>1.49</td>
<td>48.68 ± 3.43</td>
<td>12.26 ± 5.13</td>
<td>1.89±0.14</td>
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<tr>
<td><em>F. michaewskyi</em> (W)</td>
<td>45.15</td>
<td>±1.68</td>
<td>36.12</td>
<td>±1.22</td>
<td>1.25</td>
<td>40.76 ± 1.63</td>
<td>23.47 ± 2.24</td>
<td>1.99±0.30</td>
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<tr>
<td><em>F. michaewskyi</em> (E)</td>
<td>58.67</td>
<td>±3.89</td>
<td>41.67</td>
<td>±2.06</td>
<td>1.40</td>
<td>49.01 ± 5.66</td>
<td>16.72 ± 5.51</td>
<td>2.04±0.19</td>
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<tr>
<td><em>F. albaryana</em> (W)</td>
<td>49.68</td>
<td>±2.25</td>
<td>35.68</td>
<td>±1.79</td>
<td>1.39</td>
<td>43.63 ± 3.05</td>
<td>16.05 ± 4.08</td>
<td>2.10±0.28</td>
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<tr>
<td><em>F. albaryana</em> (E)</td>
<td>60.45</td>
<td>±2.17</td>
<td>42.79</td>
<td>±3.11</td>
<td>1.41</td>
<td>49.92 ± 3.51</td>
<td>13.99 ± 3.91</td>
<td>2.01±0.78</td>
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<tr>
<td><em>F. bithynica</em> (W)</td>
<td>43.22</td>
<td>±2.67</td>
<td>32.72</td>
<td>±3.71</td>
<td>1.32</td>
<td>47.52 ± 4.61</td>
<td>17.87 ± 4.68</td>
<td>1.96±0.17</td>
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<tr>
<td><em>F. bithynica</em> (E)</td>
<td>49.17</td>
<td>±8.31</td>
<td>34.98</td>
<td>±3.95</td>
<td>1.40</td>
<td>49.44 ± 4.21</td>
<td>15.00 ± 3.76</td>
<td>1.90±0.20</td>
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<tr>
<td><em>F. fleisheriana</em> (W)</td>
<td>49.60</td>
<td>±3.41</td>
<td>35.41</td>
<td>±5.22</td>
<td>1.40</td>
<td>44.66 ± 4.24</td>
<td>15.00 ± 5.18</td>
<td>1.77±0.17</td>
<td></td>
</tr>
<tr>
<td><em>F. fleisheriana</em> (E)</td>
<td>58.89</td>
<td>±4.77</td>
<td>42.76</td>
<td>±3.65</td>
<td>1.37</td>
<td>35.20 ± 3.65</td>
<td>18.52 ± 5.74</td>
<td>1.90±0.01</td>
<td></td>
</tr>
<tr>
<td><em>F. sibthorpiana</em> (W)</td>
<td>43.76</td>
<td>±2.27</td>
<td>30.68</td>
<td>±2.82</td>
<td>1.42</td>
<td>47.96 ± 2.40</td>
<td>12.24 ± 3.76</td>
<td>1.84±0.29</td>
<td></td>
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<tr>
<td><em>F. sibthorpiana</em> (E)</td>
<td>66.81</td>
<td>±2.98</td>
<td>36.17</td>
<td>±2.90</td>
<td>1.84</td>
<td>70.40 ± 3.71</td>
<td>11.04 ± 3.10</td>
<td>1.92±0.15</td>
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<tr>
<td><em>F. minima</em> (W)</td>
<td>46.27</td>
<td>±1.43</td>
<td>37.48</td>
<td>±1.91</td>
<td>1.23</td>
<td>41.14 ± 1.87</td>
<td>23.76 ± 2.22</td>
<td>1.85±0.23</td>
<td></td>
</tr>
<tr>
<td><em>F. minima</em> (E)</td>
<td>59.43</td>
<td>±3.08</td>
<td>40.98</td>
<td>±1.83</td>
<td>1.45</td>
<td>46.13 ± 4.27</td>
<td>14.19 ± 3.26</td>
<td>1.93±0.10</td>
<td></td>
</tr>
<tr>
<td><em>F. armena</em> (W)</td>
<td>46.27</td>
<td>±2.40</td>
<td>33.74</td>
<td>±1.38</td>
<td>1.37</td>
<td>41.11 ± 2.64</td>
<td>15.36 ± 4.97</td>
<td>1.90±0.15</td>
<td></td>
</tr>
<tr>
<td><em>F. armena</em> (E)</td>
<td>48.55</td>
<td>±4.36</td>
<td>36.20</td>
<td>±3.07</td>
<td>1.34</td>
<td>42.86 ± 4.19</td>
<td>13.30 ± 3.09</td>
<td>1.88±0.06</td>
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<tr>
<td><em>F. elwesi</em> (W)</td>
<td>42.75</td>
<td>±1.83</td>
<td>33.60</td>
<td>±1.66</td>
<td>1.27</td>
<td>38.29 ± 2.47</td>
<td>18.53 ± 4.56</td>
<td>1.85±0.27</td>
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<tr>
<td><em>F. elwesi</em> (E)</td>
<td>54.82</td>
<td>±3.81</td>
<td>37.44</td>
<td>±3.19</td>
<td>1.46</td>
<td>49.16 ± 3.32</td>
<td>11.52 ± 4.07</td>
<td>1.92±0.08</td>
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</tbody>
</table>


As a result of TEM examinations, we also observed that endexine is very thin in all examined taxa (Figs. 59–62). Ectexine is thicker in *Fritillaria* pollen grain in comparison to
Asparagus pollen grains. While endexine is very thin and uninterrupted in *A. verticillatus* it is interrupted in *A. palaestinus*, *F. whitallii*, and *F. armena*. The perforation number is observed to be the greatest in *A. lycicus* and in *F. armena*. In the investigated taxa, exine sculpture is reticulate, reticulate-rugulate, striate-reticulate, retipilate and suprareticulate (Figs. 23-58). El-Ghazali (1993) explained that *Asparagus flagellaris* pollen had psilate exine sculpture. However, we haven’t observed such an exine sculpture in *Asparagus* pollens. Reticulate, microreticulate, macroreticulate, faveolate-plicate, and tuberculate exine structure were mentioned in previous studies of *Fritillaria* pollen grains (Radulescu 1973, Schulze 1980, Kosenko 1991a, 1999). In this study it is detected that reticula are rather big in *Fritillaria* pollens in comparison with the *Asparagus* taxa (Figs. 23-36). According to Kosenko (1991a, 1992) small reticulae are more plesiomorphic characteristics than big ones. Kosenko (1991a) and Radulescu (1973) had grouped *Fritillaria* pollen grains according to the density of reticula. Schulze (1982) found out that the diameter of the lumina was under 1µm or approximately 2µm in *Asparagus*. During the present study it was found that the diameter of lumina was 0.10-0.55µm in *Asparagus* pollens. Schulze (1980, 1982) stated that the diameter of reticula were 1-4 µm in *Fritillaria*. In the present study, it was observed that the diameter of lumina is approximately 0.17-0.97 µm in *Fritillaria* pollens.

Ornamentational characteristics of pollen grains of the investigated taxa as observed in SEM micrographs have been used for their diagnostic features and accordingly both *Asparagus* and *Fritillaria* are proposed to be divided into four types as shown below:

**Index**

**Asparagus**

Type I: Ornamentation is reticulate:  *A. acutifolius*

Type II: Ornamentation is rugulate:  *A. verticillatus* and *A. persicus*.

Type III: Ornamentation is retipilate:  *A. lycanicus*

Type IV: Ornamentation is reticulate-rugulate:  *A. officinalis*, *A. lycicus*, *A. coodei*, and *A. palaestinus*
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**Fritillaria:**
Type I: Ornamentation is suprareticulate: *F. sibthorpiana, F. aurea, F. acmopetala subsp. wendelboi* and *F. michailovskyi.*
Type II: Ornamentation is reticulate: *F. whitallii, F. crassifolia subsp. crassifolia, F. albunya, F.minima* and *F. elwessii.*
Type III: Ornamentation is rugulate-reticulate: *F. bithynica* and *F. armena.*
Type IV: Ornamentation is striate-reticulate: *F. flesheriana.*

**Acknowledgements**
The authors thank the collectors for allowing them to use their specimens.

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(Manuscript received on 21 May, 2007; revised on 24 July, 2007)