Diameter, Exine Thickness, and Sculpturing in genera Scorzonera L., Sonchus L. and Tragopogon L. of Asteraceae in Pakistan

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Abstract: A survey of pollen morphology of 13 species representing 3 genera of tribe Lactuceae (Asteraceae) from Pakistan, have been investigated first time by Scanning Electron Microscopy. The grains are echinate, trizonocolporate, semiangular to semilobate in polar view and prolate-spheroidal to subspheroidal and spheroidal in equatorial view. The range of equatorial diameter varies from 25 µm to 50.5 µm; polar diameter varies from 24 µm to 54 µm; P/E ratio ranges from 0.8 to 1.2. The spine length varies from 1 µm to 6 µm and spine rows between colpi varies from 3 to 16.

Keywords: Pollen, Scorzonera L., Sonchus L., Tragopogon L., Lactuceae, Asteraceae

1. Introduction

This paper is the first in the series on the structural palynology of Scorzonera L., Sonchus L. and Tragopogon L. (Lactuceae) from Pakistan. The present study is a detailed account of palynology from tribe Lactuceae. Here three genera are included; Scorzonera L. (5 sp.), Sonchus L. (6 sp.) and Tragopogon L. (2 sp.). Interest in pollen morphology has increased as its useful application in systematics, paleoecology, paleobotany and inhalant allergy has been increasingly recognized. Pollen morphologists have responded to the need, created by this widespread application, for a more critical comparative analysis of pollen wall structure, and for an expansion in the number of recognized systematically and palynologically significant wall characteristics. In this response, successful use has been made of phase and ultraviolet microscopy in addition to more sophisticated light microscopy. The excellent review of pollen wall analysis [1-2] for a demonstration of the results of ultraviolet microscopy and [3], whose study of pollen walls in the Asteraceae is of real systematic value.

Sonchus asper (L.) Hill, Asteraceae is a cosmopolitan annual herb, less abundant in the tropics than in cool and temperate regions, native to the old and introduced into the new world [4,5]. Tragopogon L. (Asteraceae) comprises approximately 150 species native to Eurasia. Most species are diploid (2n=12) but some polyploidy species or cytotypes have been reported [6]. Tragopogon dubius Scop. a biennial composite. T. dubius Scop. spends its 1st yr. as a low rosette; during its 2nd yr. if its root crown diameter is sufficiently large it sends up a long stem, which eventually produces 1-30 flowering stalks [7]. Since Scorzonera L. was revised by Chamberlain for the Flora of Turkey [8], three new species and one variety have been described from Turkey: Scorzonera longiana Sumbul [9], Scorzonera pisidica Hub.-Mor., Scorzonera sandrasica Hartvig and Strid and Scorzonera latifolia (Fisch. and Mey.) DC. Var. angustifolia Pripliko apud Lipsch. [10], with the description here of Scorzonera gokcheoglui O. Ünal and R. S. Göktürk the number of species in Turkey is now 43.

2. Materials and Methods

Pollen was removed from herbarium (ISL) sheets with relevant taxonomic and collecting data provided in Table 1. Collections were made from different sites, given in the map (Fig. A). General preparation consisted of acetolyzing mature pollen grains.
[11], removal of undigested plant debris [12,13] and separating the samples for SEM as outlined below.

**SEM Preparation:**

Whole pollen grains and fragments of pollen walls obtained by cryomicrotomy [14] were stained and dried using the repeat method of osmium and thiocarbohydrazide (i.e., OTOTO) as described by [15]. Pollen was then mounted on double-stick tape and pulse sputter coated for 3 min with a gold/palladium target in a Hummer VI Sputter Coating System [13]. Secondary electron imaging and photography were done with a JEOL 880 scanning electron microscope equipped with a lanthanum hexaboride gun operating at 15-keV accelerating voltage.

### Table 1: Pollen examined of Scorzonera, Sonchus and Tragopogon from Pakistan, Herbarium (ISL)

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Collector</th>
<th>Accession number</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scorzonera ammophila</em> Bunge</td>
<td>Shaukat and Nisar</td>
<td>36273</td>
<td>Hazara</td>
</tr>
<tr>
<td><em>S. hondae</em> Kitam.</td>
<td>Iqbal Dar and M. Arif</td>
<td>35763</td>
<td>Quetta</td>
</tr>
<tr>
<td><em>S. laciniata</em> L.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. picridioides</em> Boiss</td>
<td>Muqarrab Shah and Dilawar</td>
<td>54508</td>
<td></td>
</tr>
<tr>
<td><em>S. virgata</em> DC.</td>
<td>Mir Ajab Khan and Afzal</td>
<td>57037</td>
<td></td>
</tr>
<tr>
<td><em>Sonchus arvensis</em> L.</td>
<td>Mir Ajab Khan and Manzoor Hussain</td>
<td>45508</td>
<td>Muzaffar Gar</td>
</tr>
<tr>
<td><em>S. asper</em> (L.) Hill</td>
<td>Mir Ajab Khan and Maqsood Ahmad</td>
<td>85167</td>
<td>Hazara</td>
</tr>
<tr>
<td><em>S. maritimus</em> L.</td>
<td>Muqarrab Shah and Dilawar</td>
<td>59779</td>
<td>Chitral</td>
</tr>
<tr>
<td><em>S. oleraceus</em> L.</td>
<td>Mir Ajab Khan</td>
<td>71168</td>
<td>Mansehra</td>
</tr>
<tr>
<td><em>S. palustris</em> L.</td>
<td>Manzoor Hussain and Maqsood Ahmad</td>
<td>70973</td>
<td>Sibi</td>
</tr>
<tr>
<td><em>S. uliginosus</em> M. Bieb.</td>
<td>Muqarrab Shah and Dilawar</td>
<td>56927</td>
<td>Chitral</td>
</tr>
<tr>
<td><em>Tragopogon dubius</em> Scop.</td>
<td>Mir Ajab Khan and Nisar Abbasi</td>
<td>95408</td>
<td>Skardu</td>
</tr>
<tr>
<td><em>T. gracilis</em> D. Don.</td>
<td>Mir Ajab Khan</td>
<td>108924</td>
<td>Malakand Agency</td>
</tr>
</tbody>
</table>

![Fig. A: Location of Species sampling sites in Pakistan.](image)
3. Results and Discussion

The results of the pollen investigation are summarized in Table 2 and SEM are given in Figure 1-18. The cluster analyses of different species are given in Figure 19. Systematic and evolutionary themes based on Asteraceae pollen morphology were definitively set forth by Wodehouse in publications from [16,1], although from an historical perspective, it is noteworthy that Steetz [17] is credited as the first to employ pollen as a taxonomic character in the Asteraceae [18-20]. Wodehouse recognized four pollen morphological forms in the family: simple echinate, sub-echinolophate, echinolophate, and psilolophate together with many intermediates [1], one morphological form to be present in genera Scorzonera L., Sonchus L. and Tragopogon L. of tribe Lactuceae. Blackmore’s [21] defining study of lophate pollen recognized, minimally, 23 different pollen types in the Asteraceae, and lophate patterning was most variable. The ultraviolet microscopy [3] provided the foundation for interpreting structural types throughout the family. Robinson significantly extended the pollen morphological / taxonomic database [22-32].

The influence of pollen morphology in Asteraceae systematics is understood by his comments [19], “…pollen is one of a series of characters such as stylar bases and anther appendages, observable with the compound microscope, that prove useful in delimiting natural groups. The point has been reached where I believe every taxonomic treatment in the Lactuceae should include mention of pollen type”. Later he reinforced these feelings by stating “The pollen is one of the most useful characters available, and it is inexplicable that taxa would be described at this time without detailed description of the pollen”. Similarly, [33] concluded, “Within the tribe, pollen is a good character for delimiting sections, subsections and series”. Finally the most contemporary review of pollen literature, which extended the impressive bibliography assembled, is that of the late Gamal El-Ghazaly and collaborators [34] in their introduction to their taxonomic study of Baccharoides pollen. Based on the sculpturing all pollens were echinate in Scorzonera L., Sonchus L. and Tragopogon L. The pollen classes were trizonocolporate in all taxa except in Tragopogon gracilis D. Don., trizonocolporate and tetrazonocolporate both classes were observed. There is a great variation of pollen size in Scorzonera L., Sonchus L. and Tragopogon L. Dimension of the polar axis ranged from 24 µm (Scorzonera laciniata L.) to 54 µm (Scorzonera ammophila Bunge), and equatorial axis varied between 25 µm (Sonchus maritimus L.) and 50.5 µm (Scorzonera hondae Kitam.). The ratio of polar to equatorial axis (P/E) varies between different taxa as 0.8 (Tragopogon gracilis D. Don.) and 1.2 (Tragopogon dubius Scop.). The exine thickness ranged from 1.5 µm (Sonchus palustris L.) to 13 µm (Scorzonera ammophila Bunge). The spines are present in all taxa. The character of pollen spine shows an impressive variation which is of significance at the specific and generic level and has also been helpful to understand the process of spine evolution within the tribe Lactuceae. Spine length differed considerably among all species. It ranged from 1 µm (Sonchus asper (L.) Hill) to 6 µm (Scorzonera hondae Kitam.). Number of spine rows between colpi considerably differed in the Scorzonera L., Sonchus L. and Tragopogon L. It varies from 3 (Sonchus asper (L.) Hill) to 16 (Scorzonera laciniata L.).
Fig. 7-12. Scanning Electron Micrographs (SEM) of pollen grains of Pakistan. 7. Scorzonera virgata, polar view; 8. Sonchus arvensis, polar view; 9 Sonchus asper, subpolar and subequatorial view; 10. Sonchus maritimus, polar view; 11. Sonchus palustris, subpolar view; 12. Sonchus oleraceus, polar and equatorial view.
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Fig. 19: Tree diagram for 8 variables of single linkage
Table 2: List of species examined, measurements of equatorial diameter, polar diameter, P/E ratio, exine thickness, spine length, spine rows, shape and sculpturing features in genera Scorzonera L., Sonchus L. and Tragopogon L. of tribe Lactuceae.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Taxon</th>
<th>Equatorial Diameter (µm)</th>
<th>Polar Diameter (µm)</th>
<th>P/E</th>
<th>Exine Thickness (µm)</th>
<th>Spine Length (µm)</th>
<th>Number of Spine rows b/w colpi</th>
<th>Shape in polar view</th>
<th>Shape in Equatorial view</th>
<th>Aperture type</th>
<th>Pollen Class</th>
<th>Sculpturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Scorzonera ammophila Bunge</td>
<td>42 – 46</td>
<td>44 – 54</td>
<td>1.1</td>
<td>7 – 13</td>
<td>2.7 – 5.8</td>
<td>10 – 12</td>
<td>Semiangular</td>
<td>Prolate – spherical</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>2.</td>
<td>Scorzonera hondae Kitam</td>
<td>32.5 – 50.5</td>
<td>40.5 – 48.8</td>
<td>1.1</td>
<td>4.7 – 7.4</td>
<td>2 – 6</td>
<td>10 – 14</td>
<td>Semiangular</td>
<td>Prolate – spherical</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>3.</td>
<td>Scorzonera laciniata Bunge</td>
<td>29 – 35</td>
<td>32.6 ± 0.9</td>
<td>0.9</td>
<td>1.7 – 3</td>
<td>2 – 3.4</td>
<td>12 – 16</td>
<td>Semiangular</td>
<td>Prolate – spherical</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>4.</td>
<td>Scorzonera picridioides Boiss</td>
<td>32 – 42.5</td>
<td>36 – 48</td>
<td>1.1</td>
<td>2.3 – 5.5</td>
<td>3.5 – 5</td>
<td>10 – 12</td>
<td>Semiangular</td>
<td>Prolate – spherical</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>5.</td>
<td>Scorzonera virgata Bunge</td>
<td>34 – 41.5</td>
<td>36 – 43.5</td>
<td>1.1</td>
<td>1.5 – 2.4</td>
<td>4 – 4.6</td>
<td>12 – 15</td>
<td>Semiangular</td>
<td>Prolate – spherical</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>6.</td>
<td>Sonchus arvensis L.</td>
<td>26 – 34.5</td>
<td>30.5 ± 1.5</td>
<td>1.0</td>
<td>6 – 9</td>
<td>1.5 – 3</td>
<td>5 – 6</td>
<td>Semilobate</td>
<td>Prolate – spheroidal</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>7.</td>
<td>Sonchus asper (L.) Hill</td>
<td>29 – 46.5</td>
<td>36.1 ± 3</td>
<td>0.9</td>
<td>3 – 7</td>
<td>1 – 3.5</td>
<td>3 – 7</td>
<td>Semilobate</td>
<td>Spheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>8.</td>
<td>Sonchus maritimus L.</td>
<td>25 – 35.5</td>
<td>30.3 ± 1.5</td>
<td>1.0</td>
<td>2.5 – 3.7</td>
<td>1.5 – 3</td>
<td>6 – 8</td>
<td>Semilobate</td>
<td>Subspheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
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<tr>
<td>9.</td>
<td>Sonchus oleraceus L.</td>
<td>27 – 40.5</td>
<td>32 – 40.5</td>
<td>1.1</td>
<td>7 – 10</td>
<td>1.5 – 3</td>
<td>4 – 5</td>
<td>Semilobate</td>
<td>Spheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>10.</td>
<td>Sonchus palustris L.</td>
<td>30 – 32.5</td>
<td>33.9 ± 2.2</td>
<td>1.0</td>
<td>2 – 2.5</td>
<td>1.2 – 2.2</td>
<td>5 – 8</td>
<td>Semilobate</td>
<td>Spheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>11.</td>
<td>Sonchus uliginosus M. Bieb.</td>
<td>32 – 42</td>
<td>37.4 ± 1.5</td>
<td>0.9</td>
<td>2.5 – 5.3</td>
<td>1.5 – 3.5</td>
<td>6 – 7</td>
<td>Semilobate</td>
<td>Spheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>12.</td>
<td>Tragopogon dubius Scop.</td>
<td>35 – 43.5</td>
<td>39.7 ± 1.3</td>
<td>1.2</td>
<td>6 – 10</td>
<td>3.5 – 5</td>
<td>8 – 9</td>
<td>Semilobate</td>
<td>Spheroidal</td>
<td>Nonlacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>13.</td>
<td>Tragopogon gracilis D. Don.</td>
<td>42 – 43</td>
<td>42.5 ± 0.2</td>
<td>0.8</td>
<td>5 – 7</td>
<td>2.5 – 4</td>
<td>4 – 8</td>
<td>Rectangular to Semilobate</td>
<td>Spheroidal</td>
<td>Lacunate to non lacunate</td>
<td>Tri or tetra zonocolporate</td>
<td>Echinate</td>
</tr>
</tbody>
</table>

* + = Standard error
Turkey, in particular the southwest and east of the country, is one of the main centers of diversity for the genus Centaurea L. [35]. The pollen classification of Wagenitz has been contested by some authors. A review of the structure of their pollen grains appears timely, especially because most studies of Centaurea L. pollen have been based on light microscopy. The Centaurea L. represents an artificial conglomeration of species has long been known, but its sheer complexity has to date thwarted attempts to achieve a completely natural partition of its components. Following [36] description of eight pollen types in the subtribe Centaureinae, all subsequent studies confirmed that pollen type is one of the most reliable characters for analyzing the phylogeny of Centaurea L. sensu lato. According to Wagenitz, [37] hypothesized evolutionary hierarchy; the pollen types Serratula L., Crupina (Pers.) DC., and Centaurea centaurium L. are the most primitive. The types Dealbata Willd., Cyanus Mill., and Montana L. are intermediate and the more advanced types are Centaurea scabiosa L. and Jacea L. pollen.

Another system, also based on SEM of pollen, emphasized the features of the germinal pore in equatorial view [38]. This system which is little used is numerical. Because the pollen orientations used in these systems were strikingly different, the resulting pollen types were sometimes difficult to equate. Whatever the terminological system used to describe echinolophate pollen sculpturing is unimportant. Robinson [29] believed that the lophate forms were pleisiomorphic. He further stated “It is personally believed from these studies that the lophate forms of pollen in the Lactuceae and related Vernonieae, have a common origin and that the pollen in both tribes is probably derived repeatedly by some sort of reversion”.

As the pollen morphology results indicate that pollen grains in Scorzonera L., Sonchus L. and Tragopogon L. are lophate. The spines are more or less similar in all cases, except for differences in their size and distribution. Wodehouse [1] reported that pollen grains of Compositae were unique and true to form and he outlined the principles of morphological evolution of spine form of the family in which he suggested the reduction series from long to minute spines. There seems to be a potential indicating the evolutionary processes of pollen spines in the Lactuceae. The peculiar spine character perhaps represents a climax in the apertural evolution. The occurrence of spines and its absence indicate a trend of evolution of spine reduction in the tribe Lactuceae. The reduction and the absence of spines is an evolved character in Lactuceae and the genera with spinate pollen as in Scorzonera L., Sonchus L. and Tragopogon L., indicate primitive feature as compared to the genera with spineless pollen which are considered as advanced features within the tribe. The data may be used in establishing relationship at the generic and specific level of the tribe Lactuceae within the family Asteraceae.

4. Conclusion

In view of this, it could be concluded that pollen morphology can not be solely used as the base of taxonomic classification of the Asteraceae. However, if it is accepted that pollen morphology shows evolutionary sequences comparable to those in other organs, than it may need to be given as much weight as any other morphological character. The lophate sculpturing characterize Lactuceae pollen, although this study is highly restrictive, the morphological characterizations of echinolophate sculpturing for Scorzonera L., Sonchus L. and Tragopogon L. have application to the tribe as a whole and to the entire family.

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