Palynological Study of the Genus Tragopogon from Pakistan

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Abstract: Pollen morphology of two different species, Tragopogon dubius and Tragopogon gracilis, belonging to genus Tragopogon of family Asteraceae was studied from Pakistan. Morphology of pollen grains of each of the species is based on specimens selected at random. Proposed characters i.e. grain, shape of pollen grain, equatorial diameter, polar diameter, micropyrene, polar view, equatorial diameter (P), polar diameter (P), P / E ratio, length of colpus, exine surface, exine thickness, inter poral distance, inter spinal distance, inter spinal outline, length of spines, number of spines between colpi in each species were recorded for comparison. At species level, micromorphological differences and distribution of surface pattern, shape and size of pollen have been found to exist. The pollen grains are consistently echinulate, trizonocolporate but in Tragopogon dubius pollen grains are tetrazonocolporate. Maximum, equatorial view, polar view, equatorial diameter (E), polar diameter (P), P / E ratio, length of colpus, exine surface, exine thickness, inter poral distance, inter spinal distance, inter spinal outline were observed in and Tragopogon gracilis and length of spines is maximum in Tragopogon dubius. This study demonstrates the potential of pollen studies in distinguishing some taxonomic groups in the Asteraceae.

Key words: Palynology, Tragopogon, pollen grain, Asteraceae

Introduction
Pollen morphology of the Lactucae (Cichoraeae) is probably the more distinctive tribe in the family Asteraceae. The ligulate corolla, milky sap and echinocolporate pollen form a unique combination of characters which it can be readily distinguished from the rest. This tribe consists of about 70 genera and 2300 species (Tomb, 1977). Stebbins (1953) proposed a natural system of classification for this tribe using geographical distribution, pollen morphology and chromosomal data in addition to traditional morphological characters. This method produced eight subtribes (I) Scolyminae, (ii) Cichorinae, (iii) Microserinae, (iv) Stephanomeriinae, (v) Dendrocsideoideae, (vi) Scorzonereae, (vii) Leontodontinae and (viii) Cripodinae. Jeffrey (1965) revised Stebbins's classification recognizing the importance of microcharacters like length of collector hairs on the style, shapes of hairs on stigmatic surfaces and pubescence on the corolla tube. He divided this tribe into five groups, eleven subgroups, and 23 series.

Many workers regard pollen grains of Lactucae as "Ligulilatase-type" (Faegri and Iversen, 1975; Moore and Webb, 1976) and contrast this with a "Tubuliflorae-type" for most of the remainder of the family Asteraceae. Wodehouse (1928,1935) examined a large number of taxa of this tribe primarily in an effort to formulate phylogenetic trends within the tribe. His studies led to the characterization of several basic echinocolporate patterns common in the Lactucae. Pausinger (1961) divided the tribe into two main types based on pollen characters. His Leontodon type was characterized by the possession of poral lacunae, and this Tragopogon type of the lack of poral lacunae and the aboral cones communicating to form long lacunae. While working on the comparative pollen morphology of Sonchus, Fournou (1960) found that this genus was closely related to Lasuinae. Tomb et al. (1974) studied the pollen morphology of Stephanomeriinae and showed that pollen grains of most of the tribe were echinophlate or tricolporate with same, or almost the same, number and shape of lacunae and demonstrated strikingly different exine stratification in several genera. Feuer (1974) examined the pollen grains of Microserinae, which, in contrast with the Stephanomeriinae, were predominantly echinocolporate. Skvarla et al. (1977) summarized much that was taken about pollen structure in the Asteraceae, where two major pollen types were categorized, namely, anemophilous and heliophilous, with various subtypes. Taxonomic, evolutionary and functional studies of the Asteraceae pollen grains on the basis of ultrastructure and sculpture were made by Bolick (1978), who noted two basic exine patterns: the Cavate heliophilous and non-cavate Anthemid. El Ghazali (1980) studied the pollen grains of 36 species of the subtribe Hypochoerinae. Regarding the sub tribe Scorzonereae with reference to its taxonomic significance, Blackmore (1982) recorded that the pollen grains can be distinguished by key constructed on the basis of the number and arrangement of the lacunae of the grains. Blackmore (1984) further dealt with pollen morphology of a large number of taxa of the tribe Lactucae and recognized seven distinct pollen types, which were further subdivided into smaller groups on the basis of distinguishing characters. According to Clark et al. (1980) pollen grains of the Asteraceae have been characterized as basically heliophilous, spherical or slightly flattened, tricolporate, and uniformly echinate, having internal foramina, with varying proportions of abnormalities in size and colpus number (Wodehouse, 1930, 1935; Skvarla and Turner, 1968; Skvarla et al., 1977). Pollen of the few genera that have not been difficult or impossible to distinguish by light or electron microscopy (Skvarla et al., 1977). However, in conjunction with systematic studies of Haploppappus and related genera in the subtribe Solidagininae, we have found a few cases of significant variation in pollen size, spine length, and the number of spine roofs between colpi. These characters indicate a potential for utilizing pollen characters in at least some systematic studies in the Asteraceae.

According to Larson and Lewis (1961), interest in pollen morphology has increased as its useful application in systematics, paleoecology, palaeobotany and inherent 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 phylogenetically significant wall characteristics. In this response, successful use has been made of phase and ultraviolet microscopy in addition to more sophisticated light microscopy. The reader is directed to the excellent review of pollen wall analysis in Wodehouse (1935) to Erdtmann (1956) for a demonstration of the results of ultraviolet microscopy and to Stix (1960), whose study of pollen walls in the Compositae is of real systematic value.

Pollen morphology has also been much from electron-microscopic studies of sectioned pollen grains. The division of the pollen wall into extexine, endexine and intine, as described in Faegri and Iversen (1960) (a basic discussion of the pollen wall is found in this text and in Erdtmann (1956)), was observed in the pioneer electron microscopic studies of Muhlethaler (1963). However, the sections in these studies were too thick to reveal fine structural detail. During the initial period of rapid improvement of thinning, embedding and sectioning techniques in the field of electron microscopy, the electron micrographs published by Azzelius (1954, 1955, 1956) and Azzelius et al. (1954) were made from sections of sufficient thinness to reveal fine structural detail and differentiation of wall layers. Pollen characters were used by Stebbins (1963) and monographic treatments since than have used them to some extent. Wodehouse (1935) characterized the basic exomorphology of Lactucae pollen and described two basic pollen types (echinate and lophate). There have been several careful light-microscopic studies of pollen since the wodehouse study (i.e., Pausinger, 1951; Saad, 1961). However, there has not been an in-depth study of the tribe using modern electron microscopy techniques (SEM and TEM) until recently (Tomb et al., 1974; Feuer and Tomb, in prep). Subtribes (Stephanomeriinae and microserinae, respectively). These studies have shown that pollen of most of the tribe is echinophlate or tricolporate, with the same or almost the same numbers and shapes of lacunae, and demonstrated strikingly different exine stratification patterns in several genera. Pollen characters have been careful at several levels in the systematics of the tribe. For example, in the Stephanomeriinae aperture-shape supports the division of the subtribe into two phylectic lines, a division suggested by Stebbins (1953). In the ditycic genus Glycyptolepis (Stephanomeriinae), pollen morphology (internal and external) of the two species is quite different (Tomb et al., 1974). At the population level, using
herbarium material, pollen characters have been used to map hybrids and polypliods (Tomb, 1970 and Northington, 1971). Hybrids (as well as apomictic plants) usually produce a high frequency of aborted and irregular grains. Polypliods generally have larger pollen and are often tetracolpate. Among the important contributions of these studies were the observations of variation in the fine structure of the exine, the size of the wall substance particles (50-60 A), and the stratified nature of the exine. In both gymnosperms and angiosperms plants, an inner layer of the exines was observed to be laminated and less compact than the other layers. This layer was interrupted as being a part of the endexine homologous to the endexine (Erdtman, 1952, 1960) and as having phylogenetically variable. In the plants studied, pollen grains of the gymnosperms were found to have a laminated layer considerably greater in thickness than that of any angiosperms plants studied. From this observation, a reduced laminated layer was considered phylogenetically advanced. In his electron microscopic study of the pollen wall in Santalum lancea, Ehrlich (1968) presented further evidence of a laminated layer between the intine and extine layer and observed that this laminated layer made up the aperture membrane. In Ehrlich's study, this layer is interpreted as an independent wall layer, the mesine. Some pollen morphologists now recognize the pollen wall to be composed of extine and mesine. The present value of the mesine or an analogous layer is made obvious as Rowley (1959) found no evidence of a mesine in the pollen wall in the Commeniaceae. The possibility that the lack of a mesine resulted from the techniques employed by Rowley (formalin fixation, osmic staining and methanolyte embedding) is ruled out. Under the same conditions, Brown and Hardman (1936) and Rowley also reported observing a mesine in pollen of dicotyledonous plants. The fine structure and wall stratification of fossilized pollen from the Eocene has also been analyzed by electron microscopy. Ehrlich and Hall's (1959) exciting study shows that fossilized pollen walls retain structural details, and indicates that the evolutionary development of the pollen wall may be subject to direct study. Recognizing the potential systematic and phylogenetic value of the pollen wall, especially the layer presently recognized as the mesine, a comparative study of Parkinsonia acuta pollen wall was initiated. This species was chosen because, in an examination of the fine structure of the cytoplasm of this pollen, a mesine-like layer and an aperture membrane, more complex in fine structure than any previously reported, were observed. Gymnamaceae is one of the largest families of flowering plants, consisting of 620 genera and c. 10,000 species is widely dispersed in all regions of the world (Willis, 1973). In Pakistan, it is represented by 158 genera and 420 species (Stewart, 1972; Cope, 1982). Pollen grains of Gymnamaceae also do not show much deviation from this contention and has long been recognized as remarkably uniform. Therefore palynology plays a little role in the taxonomy of this family (Woodhouse, 1935; Rowley, 1960). However according to Woodhouse (1935) two characters viz., grain size and sexine pattern use of some significance. fiberglass (1957) used the grain size as a basic character to separate wild and cultivated grasses. Rowley (1960) while studying the fine structure of some of the grass pollen used the arrangement of the spinules on the extexine as a key character for delimiting various taxa. Faegi and Iversen (1964) in their study of grass pollen, found two different types of sexine i.e. scabrate and areolate. Anderson and Bartelsen (1972) and Grant (1971) also used these types to distinguish various members of the tribe Triticaceae, Zea and Triticus, respectively. Page (1978) in his scanning electron microscopic survey of grass pollen further divided these two basic types on the basis of the proximity of granules, whether they are close or widely spaced, while the fused type are differentiated on the basis of height of granules. Chaturvedi (1971) has reported 4 types of grains in Saccharum robustum (viz., (i) unilatral monocolporate, (ii) bilatral monocolporate, (iii) bilatral double grains with two pores on either side of the dumbbell shaped pollen grains (iii) single diaporate grains and (iv) double grains with single pore. According to Memon (1984), the Scanning Electron Microscope has revealed micro-morphological details of surface patterns of pollen grains, which are not resolved either by the transmission electron microscope or by an optical microscope, the scartaste character of the exine can not be as accurately studied by the scanning electron microscope as by the transmission electron microscope. The reveal advantage of the scanning electron microscope in the sectioned study of exine in the sectional study of exine lies in the realm of middle magnitude structures in three dimensions. However, the optical microscope is very useful for basic study of pollen morphology, especially for studying the surface texture of exine. It was noted that the surface pattern of pollen grains was not completely analyzed by the scanning electron microscope than the optical microscope which exaggerates some features or does not reach the full depth of focus of exine sculpture. The remarkable architecture of pollen exine is known to be distinctive for different taxa and each species retains its specific statistic which can be characterized on the basis of pollen morphology (Memon, 1985). Erdtman (1953) segregated two such morphological groups in the family Xyridaceae and referred them to a special family Alloaxaceae solely on the basis of pollen morphological characters. The pollen morphology of family Proteaceae has revealed a wide spread heterogeneity and many genera including nearly all those with large number of species often lack unique combinations of pollen characters that could distinguish them from other genera of the family. Although some tribes and sub tribes with a small number of genera show homogeneity in their pollen morphology, nonetheless, they could not be separated from one another because invariable overlapping of surface pattern, shape and size of pollen grains was present throughout the family (Memon, 1984).

Zahur et al. (1976-78) has provided a commendable quantity of basic and applied information in this line by describing the pollen grains of 486 angiosperm species. They also described the size range of pollen grains of Graminaceae. Erdtman (1961) found uniporate pollen grains of Hordeum vulgare with 3 μ diameter. Ashraf (1973) studied some medicinal plants palynologically. Wodhouse (1965) reported pollen grains ofavena barbata as an important cause of hay fever. Malik et al. (1984) studied pollen morphology of seventy-five Pakistan medicinal plants. They observed that the pollen grains are of various shapes i.e. varying from spherical to prolate and trizo-tricolporate with fusiform or narrowly obovate, colpi that are free or occasionally fused at the poles. The pollen characteristics of some medicinal plants was made by Malik et al. (1964). This seems to be first palynological contribution from Pakistan. Although Erdtman (1962) has very elaborately covered this neglected field yet regional pollen flora remained unexplored. Khan and Bhutta (1965) studied the pollen grains of honey. In a review paper Bhutta (1968) emphasized the need of this important field of science. Soon after Khan and Memon (1970) gave an account of the pollen morphology of certain Leguminous plants of Jamshoro, Sind. Taking into account the applied form of Palynology it was found desirable to produce series of papers dealing with the fundamental Palynology of the plants growing in the Punjab. The fundamental palynology will certainly help the applied aspects. According to Ali (1988), in most of the plant groups in Angiosperms at maturity, all the pollen grains are free from each other. As the prime function of the pollen grains is to provide the male gametes to the female counterpart, in order to facilitate fertilization and ultimately the formation of the seed, such as category seems logical. However, it is also well known that in some plant groups, the pollen grains do not separate at maturity and remain associated together. The term pollen aggregate is used for all types of compound pollen grains and for various types of situations where pollen grains are dispersed in groups. Char et al. (1973) reviewed the evolutionary trends in pollen organization and discussed their adaptive significance. In the present investigation, the pollen morphology of the genus Cepalis, all Pakistan, of the tribe Lactuceae, have been studied systematically. The present writers decided to contribute as much as possible and to start with different species of the genus Asteraceae. The present study was undertaken to evaluate the morphological differences in the pollen grains of family Asteraceae.

Materials and Methods

Pollen morphology: Pollen morphology of six taxa of Tribe Lactuceae of Asteraceae (Composite) from Pakistan was studied. The floras from mature capitula were extracted, either from the herbixarum specimens of Quaid-I-Azam University, Islamabad or fresh polleniferous material collected from the wild was used in this research during the year 2001.

Pollen staining preparation: Pollen grains were stained with 1-% safranine mixed in glycerin jelly. Glycerin jelly was prepared by dissolving 70gm of
gum in 42ml distilled warm water in a beaker. The beaker was placed in another metalloid pot, containing boiling water. The gelatin was stirred for 1-2 hours. After this operation, 36ml of gelatin was added in it, followed by 1gm of phenol crystals. This warm gelatin jelly was filtered using filter paper, 1%-safranine solution was poured in it in 1:1 ratio. The homogenized mixture was preserved in a vial and was used for staining the pollen grains.

Pollen grain study by light microscopy: Florets taken from herbarium specimens were kept in distilled water in petri dishes for about 24 hours so as to soften them and were then used, while fresh material was used directly. The florets were separated from capsule and were placed in a few drops of distilled water on a clean glass slide. With the help of dissecting needle, the florets were opened, the extra material was removed and the anthers were opened, the extra material was removed and the anthers were crushed to release pollen grains on the slide. Anther wall material was discarded, while excess of water was removed with filter paper. Pollens were stained with 1% safranine mixed in glycerin jelly. The slide was placed on a hot plate and when the stain had completely melted, any bubble formed was carefully removed. Cover slip was placed on the prepared pollen glycerin jelly mixture. When cooled, the glass slide was labeled and the cover slip edges were sealed with white transparent nail varnish. The prepared slides were studied under the light microscope. Eight slides of each Taxon were prepared and complete set is kept in the Plant Taxonomy Lab, Department of Biological Sciences, Quaid-I-Azam University, Islamabad. Their photographs were taken with Nikon Apochromat Microscope LMI.

For the measurements of pollen grains, following characters were noted:
- grain shape of pollen grain:
- equatorial view, polar view, dimensions:
- equatorial diameter (E), polar diameter (P), P / E ratio, length of colpus, exine surface, exine thickness, inter-polar distance, inter spinulae distance, inter spinulae outline, length of spines, number of spines between colpi.

Results
Tragopogon gracilis D. Don. (Plate 1)
Grain: Trizonocolporate
Shape of pollen grain: Spheroidal
In equatorial view: spheroidal
In polar view: Triangular
Dimensions:
- Equatorial diameter (E): 45-(43)-46.4
- Polar diameter (P): 42.5-(43)-45
- P / E ratio: 0.95-(0.95)-0.97
- Length of colpus: 5-(5)-7.4
- Exine surface: Echinulate or Spinulate
- Exine thickness: 0.5-(0.75)-1
- Inter spinulae distance: 0.8-(0.9)-1.3
- Inter spinulae outline: V shaped
- Length of spines: 3.75-(4)-4.2
- Number of spines between colpi: 8

Plate 1: Pollen light micrograph
Tragopogon gracilis D. Don.

B. Polar view
Tragopogon dubius Scop. (Plate 11)
Grain: Tetrazonocolporate
Shape of pollen grain: Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Quadrangular
Dimensions:
- Equatorial diameter (E): 56.5-(59)-60
- Polar diameter (P): 54.4-(58.4)-52
- P / E ratio: 0.99-(0.99)-1.04
- Length of colpus: 8.75-(9.4)-10
- Exine surface: Echinulate or Spinulate
- Exine thickness: 3.75-(3.82)-3.9
- Inter-polar distance: 40-(43)-45
- Inter spinulae distance: 2.5-(3.125)-3.75
- Inter spinulae outline: V shaped
- Length of spines: 2.5-(2.75)-3
- Number of spines between colpi: 10

Plate 11: Pollen light micrograph
Tragopogon dubius Scop.

A. Equatorial view
B. Polar view
Table 1: Minimum, maximum and average values of polar and equatorial diameter, P/E ratio and Colpi length of genus Tragopogon

<table>
<thead>
<tr>
<th>Characters</th>
<th>Different Values</th>
<th>Tragopogon dubius</th>
<th>Tragopogon gracilis</th>
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<td>Equatorial diameter</td>
<td>Minimum</td>
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<td>Maximum</td>
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<td>Average</td>
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<td>Polar diameter</td>
<td>Minimum</td>
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<td>Maximum</td>
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<td>P/E ratio</td>
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<td></td>
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<td>Colpi length</td>
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<td></td>
<td>Maximum</td>
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<td>Average</td>
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<td>6.7</td>
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Table 2: Minimum, maximum and average values of inter polar diameter, inter spinal distance, length of spine and spine rows b/w colpi of genus Tragopogon

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<th>Different Values</th>
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<th>Tragopogon gracilis</th>
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<td>Maximum</td>
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<td>Average</td>
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<td>Inter polar distance</td>
<td>Minimum</td>
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<td></td>
<td>Maximum</td>
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<td></td>
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<td>Inter spinal distance</td>
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<tr>
<td></td>
<td>Maximum</td>
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<td>Average</td>
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<td>Length of spines</td>
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<td>Number of spines b/w colpi</td>
<td>Minimum</td>
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Discussion

In the present investigation the genus Tragopogon characteristic pollen grains were observed. In Tragopogon gracilis the pollen grains are trizonocporate but in Tragopogon dubius the pollen grains are tetrazonocporate. The pollen grain shape in equatorial view was sphericidal to subospheroidal, polar view is triangular in Tragopogon gracilis and quadrangular in Tragopogon dubius. Equatorial diameter varies from 45-58.5 μm (minimum) and 46.4-60 μm (maximum) (Table 1). The size of polar diameter varies between 42.5-54.4 μm (minimum) and 45-62.5 μm (maximum) (Table 1). The equatorial and polar diameter is minimum in Tragopogon gracilis 45 μm, 42.5 μm respectively. In Tragopogon dubius the equatorial diameter is 60 μm and polar diameter is 62.5 μm. Colpus length is maximum in Tragopogon dubius 10 μm while least in Tragopogon gracilis 5 μm. In Tragopogon the pollen grains are echinate or spinate. In Tragopogon dubius the exine thickness maximum 3.9 μm while minimum in Tragopogon gracilis 0.5 μm (Table 2). In this research project, some more morphological parameters were considered for their application in plant taxonomy, which were found to be useful. Minimum value of interpolar distance were found in Tragopogon gracilis 25 μm but maximum value of interpolar distance were found in Tragopogon dubius 45 μm (Table 2). The maximum interspinal distance were found in Tragopogon dubius 3.76 μm while least in Tragopogon gracilis 0.6 μm. In Tragopogon the interspinal outline is V shaped. Spine length were found to be maximum in Tragopogon gracilis 4.2 μm but minimum in Tragopogon dubius 3 μm. Number of spine rows b/w colpi varies from 8-10 in different species of Tragopogon (Table 2)

Light microscopic observations could not clearly indicate the exine sculpturing. Bolick (1978) suggested that Scanning Electron Microscopic (SEM) studies should be carried out for obtaining many characters of great taxonomic importance. Not only the general morphology but also pollen morphology is of significance in species delimitation and pollen characters are correlated with morphological features (Stix, 1960). Palynology can play a very important role in solving the taxonomic problems if the pollen characters are co-related with morphological characters to become a qualitative character. It is concluded that pollen morphology can be solely used as the base of taxonomic classification of the family. However, if it is accepted that pollen morpholohy shows evolutionary sequences comparable to those in other organs, then it may need to be given as much weight as any other morphological character. On this assumption, in the pollen morphology of the species examined, some inconsistencies and alternative relationships have been suggested in correlation with the recent taxonomic classification proposed by Johnson and Briggs (1975). The general features of the Asteraceae, taken together are not repeated in other families. This gives Asteraceae (Compositae) a unique taxonomic status. To quote "Combined efforts of systematic, evolutionary and ecological studies are still necessary to help us understand the evolution of this fascinating family, Lactuceae is a tribe of Asteraceae, some plants in this tribe are of medicinal importance. Palynological studies of Lactuceae from Pakistan are carried out for the first time. The study of pollen morphology has assumed great significance in plant taxonomy and the advancements in microscopy have led to the effective use of new pollen morphological parameters for taxonomic purposes. This research project was conducted to examine the value of pollen morphology in the taxonomy of Lactuceae and if the palynological characters are correlated to the morphological features, then they have great significance in taxonomy and may be considered as base for taxonomic decisions. For structure and pattern describing different characters, terminology followed is that of Erdtmann (1969) and Nair and Lawrance (1985). For clear understanding to the taxonomic status of the species following palynological characters like grain class, shape in equatorial and polar view, equatorial and polar diameter, P/E ratio, colpus length, exine surface, exine thickness, interpolar distance, interspinal distance, interspinal outline, length of spine, number of spines b/w colpi were also considered. It is hoped that added information of pollen grains will help in taxonomic studies of Tragopogon. Among the members of Lactuceae the general pollen morphology is similar i.e. the exine surface is echinate (spinate) or echinate (spinulate): the pollen grain is trizonoocporate but in some species tetrazonoocporate.

References


Qureshi et al.: Palynology, Tragopogon, pollen grain, Asteraceae


