PALYNOLOGICAL STUDY OF THE GENUS *TRAGOPOGON* FROM PAKISTAN

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Abstract

Pollen morphology of two different species, *Tragopogon dubius* and *T.gracilis*, belonging to family Asteraceae was studied based on specimens collected within Pakistan. Characters like grain, shape of pollen grain, 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, 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 echinate, trizonocolporate but in *Tragopogon dubius* these 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 had been observed in *Tragopogon grcilis* but length of spines is maximum in *Tragopogon dubius*. The study demonstrates potential of pollen studies in distinguishing some taxonomic groups in the Asteraceae.

Introduction

Pollen morphology of the Lactuceae (Cichorieae) is probably the more distinctive tribe in the family Asteraceae. The ligulate corolla, milky sap and echinolophate pollen form a unique combination of characters which 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 viz. Scolyminae, Cichorinae, Microseridinae, Stephanomeriinae, Dendroseiidinae, Scorzonerinae, Leontodontinae and Crepidinae. Jeffrey (1966) revised Stebbin's classification recognizing the importance of microcharacters like length of collector hairs on the style, shape of hair on stigmatic surfaces and pubescence on the corolla tube and divided this tribe into five groups, eleven subgroups, and 23 series.

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Many workers regard pollen grains of Lactuceae as "Liguliflorae-type" (Faegri and Iverson, 1975; Moore and Webb, 1978) in contrast to "Tubiliflorac-type". Wodehouse (1928, 1935) examined a large number of taxa of this tribe primarily in an effort to formulate phylogenetic trends within the tribe. The studies led to the characterization of several echinolophate patterns common in the Lactuceae.

Pausinger (1951) divided the tribe into two main types based on pollen characters. The Leontodon type was characterized by possessing poral lacunae and *Tragopogon* type by lacking poral lacunae and the abporal ones leading to form long lacunae. While working on comparative pollen morphology of *Sonchus*, Boulos (1960) found that this genus was closely related to *Launaea*. Tomb *et al.*, (1974) studied pollen morphology of Stephanomeriinae and showed that pollen grains of most of the tribe were echinolophate or tricolporate were almost the same Number and shape of lacunae demonstrated strikingly different exine stratification in several genera. Feuer (1974) examined the pollen grains of Microseridinae which in contrast to stephanomeriinae, were predominantly echinolophate. Skvarla *et al.* (1977) summarized pollen structure in the Asteraceae where two major pollen types were categorized viz. Anthemoid and helianthoid 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 caveate helianthoid and non-caveate Anthemoid. El Ghazaly (1980) studied the pollen grains of 35 species of the subtribe Hypochoeridinae. Regarding the sub-tribe Scorzonerinae with reference to its taxonomic significance, Blackmore (1982) recorded seven pollen types which could be distinguished by a key constructed on the basis of the number and arrangements of the lacunae of the grains. Blackmore (1984) further dealt with pollen morphology of a large number of taxa of the tribe Lactuceae 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 *Astereae* have been characterized as basically helianthoid, spherical or slightly flattened, tricolporate, and uniformly echinate, having internal foramina, with varying proportions of abnormalities in size and colpus number (Wodehouse, 1930, 1935). However, in conjunction with systematic studies of *Haplopappus* and related genera in the subtribe Solidaginae, we have found a few cases of significant variation in pollen size, spine length, and number of spine rows between colpi. These characters indicate a potential for utilizing pollen characters in at least some systematic studies in the Astereae.

According to Larson and Lewis (1961), 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 number of recognized systematically and phylogenatically 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) and Erdtman (1959) for a demonstration of the results of ultraviolet microscopy and to Stix (1960) whose study of pollen walls in the Composite is of real systematic value.

The division of the pollen wall into ektexine, endexine and intine, as described in Faegri and Iversen (1950) was observed in the pioneer electron microscopic studies of Fernandez-Moran and Dahl (1952) and Muhlethaler (1953). However, the sections in these studies were too thick to reveal fine structural details. During the initial period of rapid improvement of fixing, embedding and sectioning techniques in the field of electron microscopy, the electron micrographs published by Afzelius (1954, 1955, 1956) and Afzelius, Erdtman and Sjostrand (1954) were made from sections of sufficient thinness to reveal fine structural details and differentiation of wall layers. Pollen characters were used by Stebbins (1953) and monographic treatments since then. Wodehouse (1935) characterized the basic exmorphology of Lactuceae 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 microseridinae, respectively). These studies have shown that pollen of most of the tribe is echinolophate or tricolporate with the same or almost the same numbers and shape of lacunae, and demonstrated strikingly different exine stratification patterns in several genera.

Pollen characters had been useful at several levels in the systematics of the tribe e.g. in the Stephanomeriinae aperture-shape supports the division of the subtribe into two phyletic lines, a division suggested by Stebbins (1953). In the ditypic genus Glyptopleura (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 plyploids (Tomb, 1970; Northington, 1971). Hybrids as well as apomictic plants usually produce a high frequency of aborted and irregular grains. Polyploids 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 ektexine, the size of the wall substance particles (50-60 A), and the stratified nature of the exine. In both gymnospermous and angiospermaous plants, an inner layer of the exine was observed to be laminated and less compact than other layers. This layer was interepted as being a part of the endexine (homologous to the endonexine (Erdtman, 1952, 1960) and as having phylogenatic value. In the plants studied, pollen grains of the gymnospermous species were found to have a laminated layer considerably greater in thickness than that of any angiospermous plants studied. From this observation, a reduced laminated layer was considered phylogenetically advanced.

In this electron microscopic study of the pollen wall in *Saintpaulia ionantha*, Ehrlich (1958) presented further evidence of a laminated layer between the intine and ektexine and also observed that this laminated layer made up the aperture membrane. In Ehrlich's study, this layer was interpreted as an independent wall layer, the mesine. Some pollen morphologists now recognize the pollen wall to be composed of ektexine, endexine, mesine and intine.

The potential systematic 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 Commelinaceae. The possibility of lacking mesine resulted from the techniques employed by Rowley is ruled out as they were the same as those used by Ehrlich. Rowley also reported observing a mesine in pollen of dicotyledonous plants.

The fine structure and wall stratification of fossilized polen from the Eocene has also been analysed by electron microscopy. Ehrlich and Halls's (1959) exciting study shows that fossilized pollen walls retain structural details, and indicates that the evolutionary development of the pollen wall may be subjected to direct study.

Recognizing the potential systematic and phylogenatic value of pollen wall, especially the layer presently recognized as the mesine, a comparative study of *Parkinsonia aculeata* 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 had been observed.

Gramineae is one of the largest family of flowering plants, comprising 620 general and cv. 10000 species widely dispersed in all the regions of the world (Willis, 1973). In Pakistan, it is represented by 158 genera and 492 species (Stewart, 1972, Cope, 1982). Pollen grains of Gramineae 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 (Wodehouse, 1935; Rowley, 1960). However, according to Wodehouse (1935) two characters viz. Grain size and sexine pattern are of some significance. Firbas (1937) used the grain size as a basic character to separate wild and cultivated grasses. Rowley (1960), while studing the fine structure of some of the grass pollen, used the arrangement of the spinules on the ektexine as a key character for delimiting various taxa.

Faegri and Iversen (1964) in their study of grass pollen, found two different types of sexine i.e. scarbrate and areolate. Anderson and Bertelsen (1972) and Grant (1972) also used these types to distinguish various members of the tribe Triticeae, *Zea* and *Tripsacum*, 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 closely or widely spaced while the fused type are differentiated on the basis of height of granules. Chaturvedi (1971) reported four types of grains in *Saccharum robustum*.

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 (1963) segregated two south American genera (*Abolboda* and *Orectanthe*) from the family Xyridaceae and referred them to a special family Abolbodaceae 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 (Memon, 1984).

Zahur *et al.* (1975-78) provided a commendable quantity of basic and applied information by describing pollen grains of 486 angiosperm species. They also described the size range of pollen grains of gramineae. Erdtman (1961) found uniporate pollen grains of *Hordeum vulgare* with 3 μ diameter.

Ashraf (1973) studied some medicinal plants palynologically. Wodehouse (1965) reported pollen grains of *Avena barbata* as an important cause of hay fever. Malik *et al.* (1964) studied pollen morphology of seventy-five Pakistani medicinal plants. They observed that the pollen grains were of various shapes i.e. varying from spheroidal to prolate with polar or lateral germinal exits. They also reported different measurements of pollens. Elisens (1986) studied morphological variations among 12 new world genera in tribe Antirrhineae (Scrophulariaceae) with light and scanning electron microscopy. Pollens from 29 American species have mean polar diameter that range from 17 to 26 μm, have a tectate structure with perforate microreticulate or reticulate surface pattern and are subspheroidal to proloate and trizono-colporate with fusiform or narrowly oblong, colpi that are free or occasionally fused at the poles.

Pollen characteristics of some medicinal plants had been studied by Malik *et al.*, (1964) which seems to be first palynological contribution from Pakistan. Although Erdtman (1952) has very elaborately covered this neglected field yet regional pollen flora remained unexplored. Khan and Bhutta (1965) studied pollen grains of honey. 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 plants growing in Punjab.

According to Ali (1988), in most of the plant groups in Angiosperms all pollen grains are free from each other at maturity. As the prime function of the pollen grains is to facilitate fertilization and ultimately formation of seed, such a category seems logical. However, it is also well known that in some plant groups pollen gains do not separate at maturity and remain associated to gether. 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, Swamy and Cheluviah (1973) reviewed the evolutionary trends in pollen organization and discussed their adaptive significance.

In the present investigations pollen morphology of the genus *Crepis*, tribe Lactuceae, and family Asteraceae have been studied systematically.

Materials and Methods

Pollen Morphology

Pollen morphology of six taxa of Tribe Lactuceae of Asteraceae (Composite) from Pakistan was studied. The florets from mature capitula were extracted either from the herbarium specimens of Quaid-I-Azam University, Islamabad or fresh poliniferous material had been collected from its native habitat.

Pollen Staining Preparation

Pollen grains were stained with 1% safranine mixed in glycerin jelly. Glycerin jelly was prepared by dissolving 70 g of gelatin in 42 ml distilled warm water in a beaker, which was placed in another metalloid pot, containing boiling water. The gelatin was stirred for 1-2 hours. After this operation, 35 ml of gelatin was added followed by 1 g of phenol crystals. This warm gelatin jelly was filtered and 1% safarinine solution was poured in 1:1 ratio. The homogenized mixture was preserved in a vial and was used for staining the pollen grains.

Method of 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 capitula and placed in a few drops of distilled water on a clean glass slide. With the help of dissecting needles, the florets were opened, the extra material was removed and the anthers were opened. 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. Pollen were stained with 1% safranine mixed in glycerin jelly. The slide was placed on a hot plate and when the stain had completely melted and any bubble formed was carefully removed. Cover slip was placed on pollen glycerin jelly mixture. When cooled, the glass slide was labelled 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 was kept in the Plant Taxonomy Lab; Department of Biological Sciences, Quaid-I-Azam University, Islamabad. The photographs were taken with Nikon Apaphot Microscope.

For the measurements of pollen grains, following characters were noted:

Grain, shape of pollen grain, equatorial view, polar view, Dimension, 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, Length of spines and Number of spines between colpi

Results and Discussion

Tragopogon gracilis D. Don.; Grain: Trizonocoplate; Shape of pollen grain : Spheroidal; In eequatorial view: spheroidal; In polar view: Triangular; Dimentions: Equatorial diameter (E): 45-(45.7)-46.4; Polar diameter (P): 42.5-(43.75)-45, P/E ratio: 0.95-(0.96)-0.97; Length of colpus: 5-(5.7)-6.4; Exine surface: Echinate or Spinate; Exine thickness: 0.5-(0.75)-1; Inter poral distance: Inter spinal distance: 0.5-(0.9)-1.3; Inter spinal outline: V shaped; Length of spines: 3.75-(4)-4.2; Number of spines between colpi: 8.

Tragopogon dubius Scop.; Grain: Tetrazonocolporate; Shape of pollen grain: Sub spheroidal, In Equatorial view: sub-spheroidal; In polar view: Quadrangular, Dimentions: Equotorial diameter (E): 58.5-(59.25)-60, Polar diameter (P): 54.4-(58.45)-62.5, P/E ratio: 0.93-(0.99)-1.04, Length of colpus: 8.75-(9.4)-10, Exine surface: Echinate or Spinate, Exine thickness: 3.75-(3.82)-3.9, Inter poral distance: 40-(43)-46, Inter spinal distance: 2.5-(3.1215)-3.75, Inter spinal outline: V shaped, Length of spines: 2.5-(2.75)-3, Number of spines between colpi: 10.

Table 1. Minimum, maximum and average value of polar and equatorial diameter, P/E ratio and colpi length

S.No.	Traits studies (units)	*T. dubius*			*T.gracilis*		
		Max.	Min.	Avg.	Max.	Min.	Avg.
1	Equatorial diameter	60.0	58.5	59.25	46.4	45.0	45.7
2	Polar diameter	62.5	54.4	58.45	45.0	42.5	43.75
3	P/E ratio	1.04	0.93	0.98	0.97	0.95	0.96
4	Colpi length	10.0	8.75	9.3	6.4	5.0	5.7

Table 2. Minimum, maximum and average values of inter polar distance, inter spinal distance, length of spine and spine rows b/w colpi.

S.No.	Traits studies (units)	*T. dubius*			*T.gracilis*		
		Max.	Min.	Avg.	Max.	Min.	Avg.
1	Exine Thickness	3.9	3.75	3.82	1.0	0.5	0.75
2	Inter Poral Distance	46.0	40.0	43.0	28.0	25.0	26.5
3	Inter Spinal Distance	3.75	2.5	3.12	1.3	0.5	0.9
4	Length of Spines	3.0	2.5	2.75	4.2	3.75	3.97
5	Number of Spines b/w Colpi		10			8	

Research 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.

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, which gives Asteraceae (Compositae) a unique taxonomic status. Lactuceae is a tribe of Asteraceae and some plants in this tribe are of medicinal importance. Palynological studies of Lactuceae from Pakistan had been 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.

For structure and pattern describing different characters, terminology followed is that of Erdtman (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, interporal 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 member of Lactuceae the general pollen morphology is similar i.e. exine surface is echinate (spinate) or echinate (spinulate). Pollen grain is trizonocolporate but in some species tetrazonocolporate.

In the present investigations the genus *Tragopogon* characteristic pollen grains were observed. 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 charcters are correlated with morphological features (Stix, 1960). Palynology can play a very important role in solving the taxonomic problems if pollen characters are correlated with morphological characters.

In the genus *Tragopogon*, characteristics pollen were observed. In *Tragopogon gracilis*, pollen grains are trizonocolporate but in *Tragopogon dubius* these are tetrazonocolporate. The pollen grain shape in equatorial view was spheroidal to subspheroidal; 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). The size of polar diameter varies between 42.5-54.4 μm (minimum) and 45-62.5 μm (maximum). The equatorial and polar diameters are minimum in *Tragopogon gracilis* 45 μm, 42.5 mm respectively. In *Trapogon 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 mm while minimum ifn *Tragopogon gracilis* 0.5 mm. Minimum value of interporal distance was found to be 25 mm in *Tragopogon gracilis* but maximum value of interporal distance was found in *Tragopogon dubius* (46 mm). Maximum interspinal distance by 3.75 mm in *Tragopogon dubius* while least in *Tragopogon gracilis* (0.5 mm). In *Tragopogon* the interspinal outline is V shaped. Spine length by 4.2 mm in *Tragopogon gracilis* but 3 mm in *Tragopogon dubius*. Number of spines rows b/w colpi varied between 8-10 in different species of *Tragopogon*.

It is concluded that pollen morphology can not be solely used as the base of taxonomic classification of the family.

References

Afzelius, B.. 1954. La structure du sporoderme vue au microscope electronique. Rapports et communications. Section 6: 241.8 Congres International de Botanique.

Afzelius, M.B. 1955. On the fine structure of the pollen wall in Clivia miniata. Bot. Notiser. 108: 138-145.

Afzelius, B.M. 1956. Electron microscope investigations into exine tratification. Grana Palynologica. 12: 22-37.

Afzelius, B.M., G. Erdtman and F.S. Sjostrand. 1954. On the fine structure of the outer part of the spore wall of Lycopodium clavatum as revealed by the electron microscope. Svensk Bot. Tidskr. 48: 155-161.

Ali, S.A. 1988. The functional significance of pollen aggregates in angiosperms. Pak. J. Bot., 20(1): 21-44.

Anderson, S. Th. and F. Bertelsen. 1972. Scanning electron microscopic studies of cereals and other grasses. Grana, Palynologica, 12: 79-86.

Ashraf, M. 1973. Pollen morphology of certain medicinal plants of Punjab plains. M.Sc. Thesis, Pb Univ., Lahore.

Bhutta, A.A. 1968. Palynology- the study of pollen and spores. Horizon, 1047-49.

Char, M.B.S., M.S.N. Swamy and M.C. Cheluviah. 1973. A critical evaluation of the adaptive significance of the monadsand compound grains in Angiosperms. J. Palynol., 9: 39-44.

Chaturvedi, M. 1971. Pollen grains of *Saccharum robustum*. Grana, 11:87-90.

Clark. W.D., G.K. Brown and R.A. Mayes. 1980. Pollen morphology of haplopappus and related genera (Compositae-Astereae). American Journal of Botany. 67(9):1391-13963.

Cope, T.A. 1982. Poaceae. In: E. Nasir and S.I. Ali (Eds.). Flora of Pakistan. 143: 1-678, Islamabad.

Ehrlich, H.G. 1958. Electron microscope studies of *Saintpaulia ionantha* Wendl. Pollen walls. Exptl. Cell Res. 15: 463-467.

Ehrlich, H.G. and J.W. Hall. 1959. The ultrastructure of Eocene pollen. Grana Palynologica 21: 32-35.

El Ghazaly, G. 1980. Palynology of Hypochoeridinae and Scolyminae (Compositae). Opera Bot. 58: 1-48.

Elisens, W.J. 1986. Pollen morphology and systematic relationship among new world species in tribe Antirrhineae (Scrophulariaceae). Amer. J. Bot. 73(9): 1298-1311.

Erdtman, G. 1959. UV micrographs and photomicrographs from the palynological laboratory, Stockholm-Solna. Grana Palynologica. 21: 36-39.

Erdtman, G. 1961. Pollen and spore morphology plant taxonomy. Pteridophyta. P. 272. Almqvist and Wiksell, Stockholm.

Erdtman, G. 1963. Palynology. In, Advances in Botanical Research. (Ed.) R.D. Preston, 1: 149-208.

Erdtman, G. 1969. Handbook of Palynology. An introduction to the study of pollen grains and spores. Munksgaar, Copenhangen.

Erdtman, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. Chronica Botanica Col., Waltham, Mass.

Grant, C.A. 1972. A scanning electron microscope survey of some Maydeae pollen. Grana, 12: 177-184.

Jeffrey, C. 1966. Notes on Compositae 1. The Cichorieae in East Tropical Africa. Kew Bull. 18: 427-486.

Johnson, L.A.S. and B.G. Briggs. 1975. On the Proteaceae- the evolution and classification of a southern family. Bot. J. Linn. Soc., 70: 83-182.

Khan, K.M. and A.A. Butta. 1965. Pollen analysis of honey. All Pak. 17th Sci. Conf., Karachi.

Khan, K.M. and H.R.A. Memon. 1970. Palynological investigations of some members of family Leguminosae from Jamshoro (Sind), West Pakistan. Pak. J. Bot. 2(1): 25-35.

Larson, Donald A. and C.W. Lewis. 1961. Fine structure of Parkinsonia Aculeata pollen. 1. The pollen wall in American Journal of Botany. 48: 934-943.

Muhlethaler, K. 1953. Untersuchungen iibr die strukture der pollenmembran. Mikroskopie (Wien.). 8: 103-110.

Nair, P.K.K. and R. Lawrernce. 1985. Pollen morphology in compositae. Kalyani Publishers. New Delhi, India.

Northington, D.K. 1971. "Taxonomy of Pyrrhopappis a cytotaxonomic and chemosystematic study". Ph.D. Dissertation, University of Texas, Austrin.

Page, J.S. 1978. The scanning electron microscope survey of grass pollen. Kew Bulletin. 32: 313-319.

Pausinger. F. 1951. Vom Blutenstaub der Wagwarten (die Pollengestaltung der Cichorieeae). Carinthia. 13: 3-47.

Saad, S.I. 1961. Pollen morphology in The Genus Sonchus. Pollen et Spores 3. 247-260.

Stix, E. 1960. Pollen morphologische untersuchungen an Compositen. Grana Palynologica. 22: 41-114.

Tomb, A.S., D.A. Larson and J.J. Skvarla. 1974. Pollen morphology and detailed structure of family compositae, tribe cichorieeae. 1 Subtribe Stephanomeriinae. Amer. J. Bot. 61: 486-498.

Tomb, A.S. 1977. Lactuceae-systemativ Review. In: V.H. Heywood, J.B. Harborne and B.L. Turner (Editors) In: Biology and Chemistry of Compositae. Academic Press, London, New York. 1067-1079.

Willis, J.C. 1973. A dictionary of the flowering plants and ferns. Cambridge Univ. Press. 1245.

Wodehouse, R.P. 1930. Pollen grains in the identification and classification of plants-V. Haplopappus and other Astereae: The origin of their furrow configurations. Bull. Torrey Bot. Club 57: 21-46.

Wodehouse, R.P. 1935. Pollen grains, their structure, identification and significance in medicine. Hafner Publishing Company, New York, pp.574.
Wodehouse, R.P. 1935. Pollen grains. McGraw Hill, New York.

Wodehouse, R.P. 1965. Pollen grains. Hanfan and Inc. New York.

Wodehouse, R.P. 1928. The phylogenetic value of pollen grain characters. Ann. Bot. 42: 891-834.