Pak. J. Bot., 36(4): 701-710, 2004.

POLLEN MORPHOLOGY AS AN AID TO THE IDENTIFICATION OF *SCORZONERA* (CICHORIEAE-COMPOSITAE) FROM PAKISTAN

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Abstract

Pollen morphological characters of 5 species of *Scorzonera* have been investigated from Pakistan by light microscopy. Pollen grains are usually radially symmetrical, isopolar, prolate-spheroidal in equatorial view, semi-angular to inter-semi-angular in polar view, trizonocolporate, non-lacunate and echinate. Pollen characters such as size, shape, colpi and exine thickness, and aperture type are found considerably important. *Scorzonera hondae* can be distinguished due to its sub-prolate shape whereas *S. picridioides* has oblate-spheroidal subprolate P/E ratio (0.95). The pollen spine character is a diagnostic character in this genus. *S. hondae* has the highest spine length (4.8 µm) and *S. laciniata* can be distinguished due to lowest spine length 2.5 µm. There is a great range of variation in exine thickness which has proved useful at specific level. On the basis of exine thickness 3 groups viz. Group 1: *S. virgata, S. laciniata,* Group II: *S. picridioides,* Group III: *S. ammophila, S.* hondae are recognized.

Introduction

Palynology is the science of pollen and spore morphology. The knowledge of palynology can be used as an instrument of multiple research for systematic botany, paleobotany, paleoecology, pollen analysis, aeropalynology, criminology, allergy, stratigraphic correlation of oil bearing rocks and coalfields, drugs and improvement of honey. It is used as a means of tracing the history of cultivated cereals (Erdtman, 1954).

The family Compositae is a typical example of eupalynous group and most of its genera possess trizonocolporate pollen (Sachdeva & Malik, 1986). The pollen grains of Compositae have been characterized as basically helianthoid, spherical or slightly flattened, tricolporate, echinate with variation in size and colpus number (Wodehouse, 1930, 1935; Skvarla *et al*., 1977). The family is one of the largest among the angiosperms with a world-wide distribution having over 20,000 species in over 1000 genera (Heywood *et al*., 1977). Various workers viz., Selling (1948, Ikuse (1956), Nair (1965), Jain & Nanda (1966), Rao & Shukla (1975), Chopra & Nair (1965), Srivastava (1976), Feuer & Tomb (1977), Vincent & Norris (1989), Cillier (1991), Hodalova & Martonfi (1995), Nakajima & Monteiro (1995), Heslop-Harrison (1969), Stanley & Linsken (1974), Reitsma (1970), Mascarenhas (1975), Ferguson & Muller (1976), Manten (1970) published pollen morphology of Compositae on various lines in their respective flora. Skvarla *et al*., (1977) have provided critical review of pollen morphology in relation to taxonomy and evolution. Nair (1972a,b) and Walker (1976) placed the highly advanced position of the Compositae in evolutionary hierarchy. The research work of (Bhutta, 1968, Malik, 1964, Siddiqua 1964, Zahur *et al*., 1975-78; Meo *et al*., 1988a b, 1989; Meo, 1999; Nasreen and Khan 1998; Mumtaz *et al*., 2000; and Dawar *et al*., 2002) have provided a commendable quantity of basic and applied information on palynology by

describing different species. The pollen morphology of *Scorzonera* have not been palynologically studied in Pakistan. Stewart (1977) has reported 22 species of *Scorzonera* from Pakistan. However, pollen morphology or description of these species is not available from Pakistan. Nair & Lawrence (1985) described pollen morphology of *Scorzonera divaricata* from India. Pollen morphology of some *Scorzonera* species of the family Compositae from Pakistan is presented herein.

Materials and Methods

The pollinferous material for the present investigation was obtained from the herbarium of Quaid-i-Azam University (ISL), Islamabad. Mature, unopened buds were removed from the herbium specimen. The pollen preparations have been made by the acetolysis methods for light microscopic study according to the procedure outlined by Erdt'man (1966). Florets were treated in acetic acid for five minutes. Pollen grains were prepared for light microscopic study by mounting in glycerine jelly and observed under Nikon Labophot microscope using oil immersion. Polar axis (P), equatorial diameter (E), P/E ratio, exine thickness, shape in polar view, shape in equatorial view, spine length, number of spine rows between colpi and aperture type were examined. Permanent slides for pollen reference collection has been deposited in the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

The terminology used are after Erdtman (1952), Faegri & Iversen (1964), Kremp (1965), Huang, (1972), Punt *et al*., (1994) and Walker & Doyle (1975).

Results

A list of specimens investigated is given in the Appendix-I. The palynological data using light microscopy have been recorded for each species (Table 1). Light microscopic micrographs of *Scorzonera* species are presented in Figs. 1-3. Quantitative and qualitative characters are tabulated from the taxa examined.

Size: The size of the pollen grains (polar-equatorial diameter excluding spines of the *Scorzonera* species ranges from 30.7-31.7 µm to 51.0-48.9 µm. There is great variation in the size of the pollen grain (Table 1).

Symmetry and shape: Pollen grains are radially symmetrical and isopolar. Outline is prolate-spheroidal in equatorial view and semi-angular to inter-semi-angular in polar view (Table 1).

Aperture: Pollen grains are trizonocolporate. The ora are circular. Aperture is non-lacunate in all the species examined (Table 1, Figs. 1-3).

Exine: Tectum is microperforate. Exine thickness ranges from 2.1-10.2 µm. Highest exine value (10.2 µm, 6.5 µm) are recorded in *S. ammophila S. hondae* and lowest values (2.1 µm, 2.4 µm) in *S. virgata* and *S. laciniata* with intermediate values (4.3 µm) in *S. picridioides.*

Table 1. Summary of pollen measurements in *Scorzonera* (Cichorieae-Compositae).

Taxon	Equatorial view µm	Polar view µm	P/E ratio	Exine width µm	Spine length µm	No. of spine rows between colpi	Shape in equatorial view	Shape in polar view	Pollen class	Aperture type	Sculpture
Scorzonera ammophila Bunge	*48.9 c (44-45.5)	51.0 c (49-52.5)	1.04	10.2 c (7.5-12.5)	4.7 ac 3.5-6)	10-12	Prolate-spheroidal	Semi-angular	Trizonocolporate	Non-lacunate	Echinate
Scorzonera hondae Kitam	39.2 abd (32.5-51.5)	46.9 d (42.5-51)	1.20	6.5 (5-7.5)	4.8 acd (4-6)	10-14	Sub-prolate-	Semi-angular	Trizonocolporate	Non-lacunate	Echinate
Scorzonera laciniata L.	31.7 b (29-36)	30.7 b (24-34)	0.97	2.4 ab (1.5-3)	2.5 b (2-3.5)	12-16	Oblate-spheroidal	Semi-angular	Trizonocolporate	Non-lacunate	Echinate
Scorzonera picridioides Boiss	42.6 acd (34-46.5)	40.3 ad (36-50)	0.95	4.3 d (2.5-6)	4.3 acd (3.5-5)	10-12	Oblate-spheroidal	Semi-angular	Trizonocolporate	Non-launate	Echinate
Scorzonera virgata DC. Prodr.	37.5 a (34-41)	40.4 a (36-45.5)	1.08	2.1 a (1.5-2.5)	4.4 a (4.0-5)	12-15	Prolate-spheroidal	Inter-semi-Angular	Trizonocolporate	Non-lcunate	Echinate

All such means which share a common letter are insignificantly different. Otherwise they differ at $P < 0.05$.
*Mean values followed by min-max in parentheses. P= Polar, E= Equitorial.

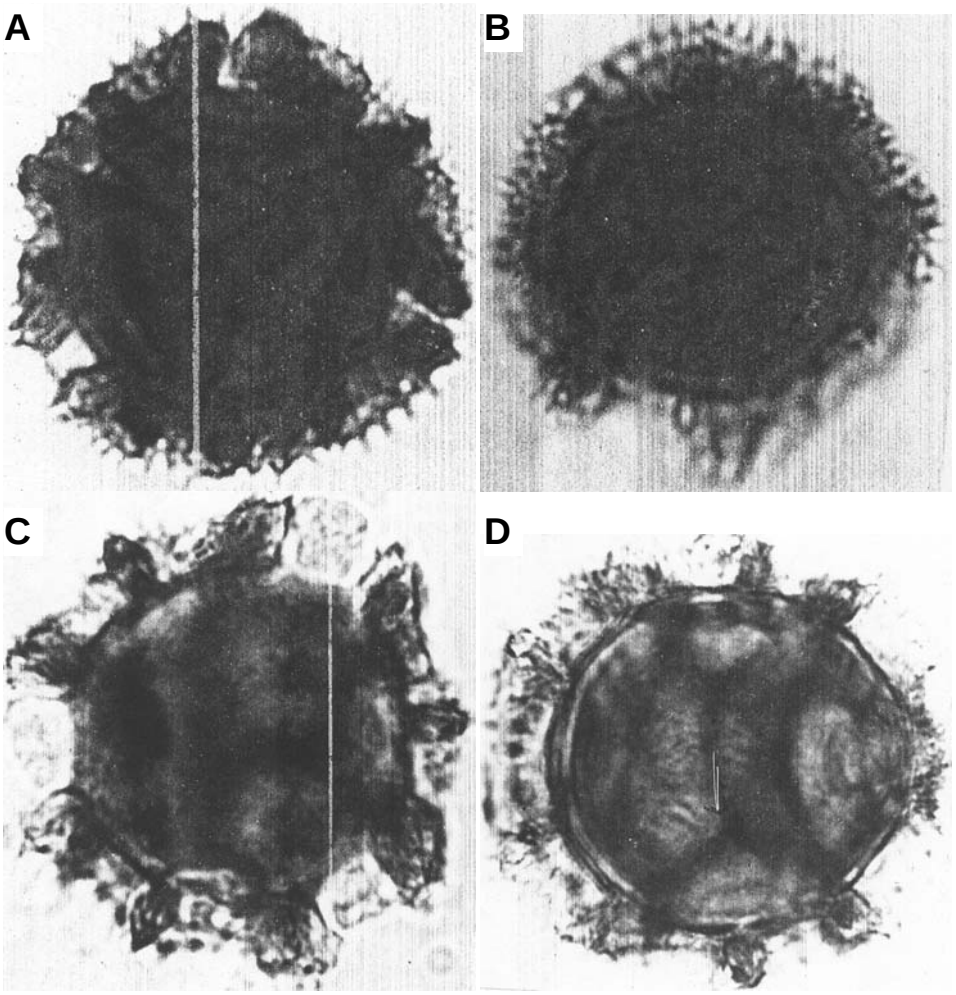

Fig. 1A-D. Light micrographs of pollen grains (X1000). *Scorzonera ammophila*: A, Polar view; B, Equatorial view. *S. hondae*: C, Polar view; D, Equatorial view.

Spine: Spines are conical with broad base. Spine length varies between 2.5 µm to 4.8 µm among the taxa. *S. hondae, S. ammophila* showed highest value. *S. virgata, S. picridioides* showed intermediate values. *S. laciniata* showed lowest value. The number of spine rows between colpi varies from 10-16. Highest number of spines found in *S. laciniata* and the lowest values are recorded in *S. ammophila, S. picridioides* respectively showing a great range of variation among the Scorzonera species. In *S. ammophila, S. hondae* spines surrounding the colpi are large while small spines are found between the large spines. Colpi are long in *S. virgata, S. hondae, S. picridioides* and *S. ammophila* while short colpi has been observed in *S. laciniata*. A key to the species of *Scorzonera* (Cichorieae) is presented for identification.

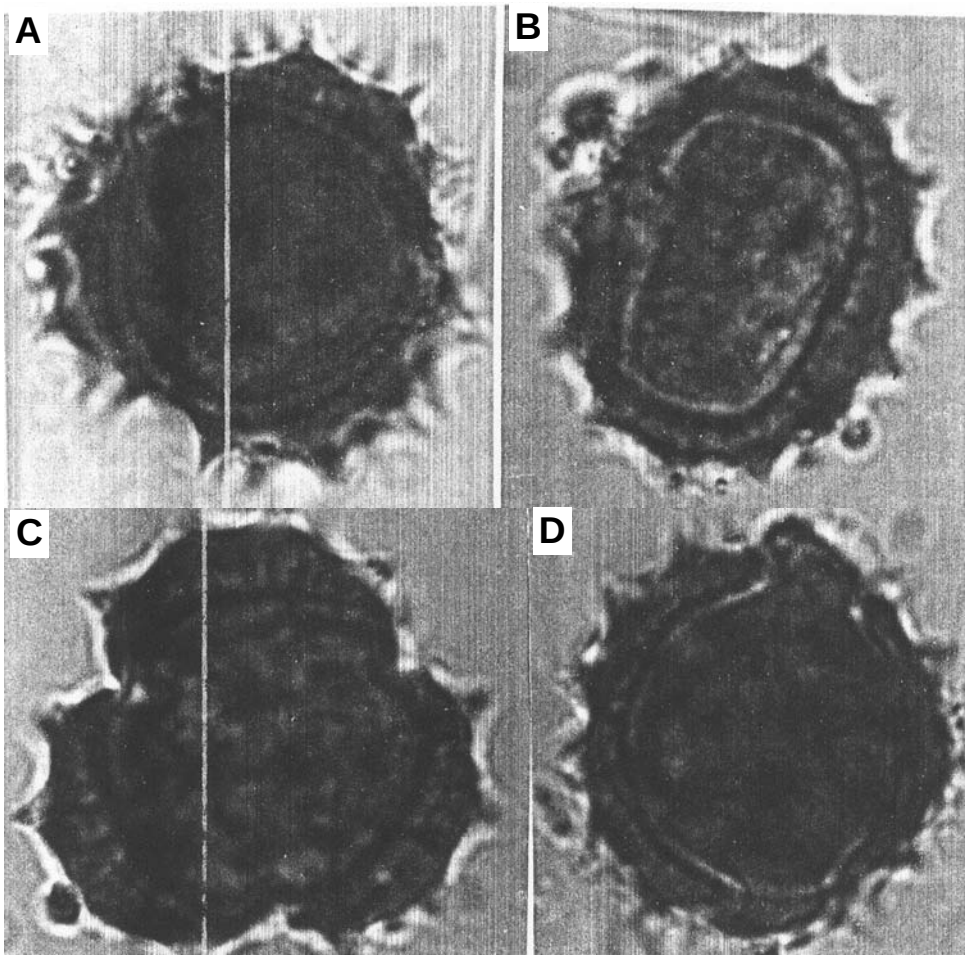

Fig. 2A-D. Light micrographs of pollen grains (X1000). *Scorzonera laciniata*: A, Polar view; B, Equatorial view. *S. picridioides*: C, Polar view; D, Equatorial view.

Key to the species of Scorzonera

1. P/E ratio 1.20 ……...…….. *Scorzonera hondae*
1. P/E ratio 0.95-0.97 ……..…….. 2
2. Pollen spine length 2.5µm …….. *S. laciniata*
2. Pollen spine length 4.4-4.8µm ……..…….. 3
3. Pollen shape inter semi-angular in polar view …….. *S. virgata*
3. Pollen shape semi-angular in polar view ...…….. 4
4. Exine thickness 4.3 µm ……... *S. picridioides*
4. Exine thickness 10.2 µm …….. *S. ammophila*

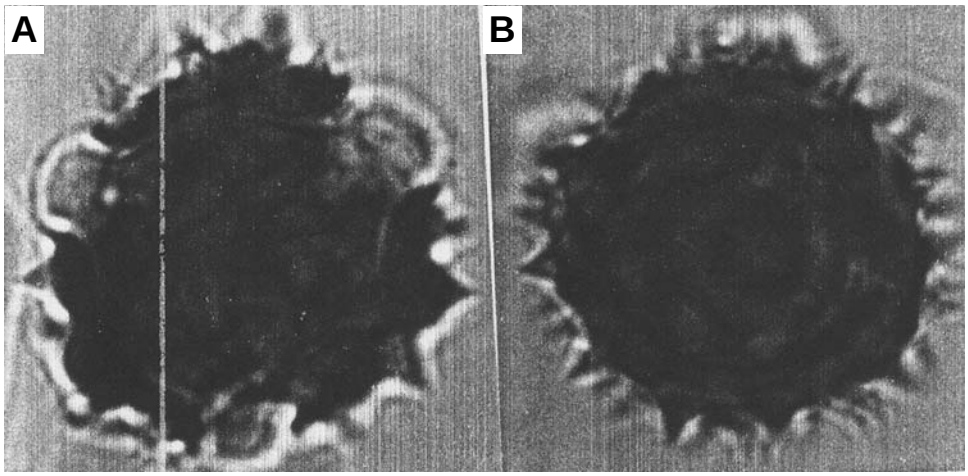

Fig. 3A-B. Light micrographs of pollen grains (X1000). *Scorzonera virgata*: A, Polar view; B, Equatorial view.

Discussion

Pollen morphology can be useful in supporting taxonomic suggestions (Clark *et al*., 1980). Application of palynology is very diverse and multidisciplinary. However, the role of pollen morphology is of significance in taxonomic debate for classification. Pollen characters have proved useful for systematic purposes in various plant families. Tomsovic (1997) utilized pollen character as an additional information for systematic studies. Huang (1972) also used pollen characters for systematic purposes. In the genus *Scorzonera*, pollen shape, exine width, P/E ratio, spine length and number of spine rows between colpi have proved useful diagnotic characters to identify the species of this genus.

S. picridioides can be distinguested due to lowest P/E ratio 0.95 (Table I). The character of pollen spine is very characteristic in the family Compositae. Hall (1928) and Clark *et al*., (1980) studied tribe Astereae (Compositae) and distinguished some genera on the basis of pollen size, spine length and number of spine rows between colpi. These characters indicates a potential for utilizing pollen characters in systematic studies in Astereae. There is a range of variation in spine length. *S. hondae* has the long spine (4.8 µm) while *S. laciniata* has short spine length (2.5 µm). Number of spine rows between colpi is also a variable character in this genus. *S. laciniata* and *S. virgata* can be placed in the same group due to similar number (12-16) while *S. hondae S. ammophila* and *S. picridioides* can be placed in same group (10-12 or 14 number). It is concluded that the charcter of spines have great potential to distinguish further taxonomic group in this genus for further taxonomie studies of the genus *Scorzonera*. Tomb *et al*., (1974) utilized spine length as an important diagnostic character in Cichorieae (Compositae) and reported that *Glyptopleureae marginata* showed a considerable reduction in spine height in comparision with *G. setulose* and other Stephanomeriinae. Dawar *et al*., (2002) applied spine length along with other pollen characters and recognized three major pollen types in *Inula* L., and its allied genera. Keeley & Jones (1977) concluded that wide variety of distinctive pollen in *Vernonia* would help to understand the basic patterns within the

Appendix 1. Specimens investigated.

Taxon	Locality	District	Voucher number	Collected by	Date
Scorzonera ammophila Bunge.	Shikwa	Chitral	2140	Muqarrab Shah & Dilawaar,	12.7.77
	Lalazar	Hazara	217	Iqbal Dar *et al*	2.9.75
	Siran	Hazara	975	Shaukat & Nisar	19.8.76
	Deosai	Skardu	25	Mir Ajab & Nisar	3.8.78
	Gattian	Hazara	887	Shoukat & Nisar	13.8.76
	Patrak	Dir	2733	Muqarrab Shah & Dilawar	8.8.77
	Totti Nullah	Skardu	2053	Mir Ajab *et al*	21.7.78
Scorzonera hondae Kitam.	Kargah	Gilgit	661	Mir Ajab & M. Afzal	15.6.77
	Aliabad	Gilgit	457	Mir Ajab & M. Afzal	6.6.77
	Nagar	Gilgit	510	Mir Ajab & M. Afzal	7.6.77
	Pishin	Pishin	631	Manzoor & Maqbool	29.4.77
	Ziarat	Sibbi	1343	Manzoor & Maqbool	28.9.77
	Orak road	Quetta	797	Manzoor & Maqbool	2.5.77
	Hanna Lake	Quetta	426	Iqbal Dar & M. Arif	12.5.76
Scorzonera laciniata L.	Sunjan	Sibi	67	Manzoor & Dilawar	6.6.78
	Ziarat	Sibi	1249	Muqarrab Shah	27.5.79
	Urrah	Quetta	563	Iqbal Dar & M. Arif	21.5.76
	Ghoilara	Quetta	677	Muqarrab Shah & Wali	12.5.79
Scorzonera picridioides Boiss.	Aspar	Kafristan	356	Muqarrab Shah & Dilawar	26.5.77
	Pashpogol	Chitral	424	Muqarrab Shah & Dilawar	28.5.77
	Pishin	Pishin	617	Manzoor & Maqbool	29.4.77
	Badamzai	Zhob	672	Manzoor & M. Arif	18.4.78
Scorzonera virgata DC. Prodr.	Skardu	Skardu	426	Awal Badshah Khan	May 1976
	Badamzai	Zhob	709	Manzoor Hussain & M. Arif	18.4.78
	Orak Lake	Quetta	947	Manzoor Hussain & M. Arif	27.4.78
	Mastung	Kalat	1182	Muqarrab Shah & Wali	25.5.79
	Kolpur	Kochi	851	Muqarrab Shah & Wali	15.5.79

genus and developed a meaningful classification at the infrageneric level. Similarly Pinar & Dönmez (2000) recorded that spine cavities of pollen exine can be regarded as diagnostic characters in the genera of Compositae. Variation in exine thickness is also prominent in this genus. The character of exine thickness in this genus can be useful at specific level since almost all the species have different exine thickness (Table 1). *S. virgata* and *S. laciniata* can be palced in the same group and *S. picridioides* can be separately placed in the second group. However, *S. ammophila, S. hondae* can be placed in the third group due to highest value of exine thickness.

References

Bhutta, A.A. 1968. Palynological study of pollen and spores. *Horizon*., 10: 47-49.

Chopra, S. and P.K.K. Nair. 1965. Palynological studies on Indian *Taraxaca* L. *Proc. Indian Acad. Sci*., Bect. B, 61: 214-221.

Cilliers, S.S. 1991. Pollen morphology and its taxonomic value in *Bachylaena* (Asteraceae) in South Africa. *S. Afr. J. Bot*., 57: 325-330.

Clark, W.D., G.K. Brown and R.A. Mayes. 1980. Pollen morphology of *Haplopappus* and related genera (Compositae – Astereae). *Amer. J. Bot*., 67: 1391-1393.

Dawar, R., M. Qaiser and A. Perveen. 2002.Pollen morphology of *Inula* L., (s.str.) and allied genera (Inuleae – Compositae) from Pakistan and Kashmir. *Pak. J. Bot*., 34(1): 9-22.

Erdtman, G. 1952. *Pollen morphology and Plant Taxonomy-Angiosperm (An introduction to Palynology*. 1: Almqvist and Wiksell. Stockholm.

Erdtman, G. 1954. *An introduction to pollen analysis* (Chronica Botanica Co: U.S.A.

Erdtman, G. 1966. *Handbook of Polynology*. Munksgaard, Copenhagen. Pp. 21-77.

Faegari, K. and J. Iversen. 1964. *Text book of pollen analysis* 2nd ed. Muksgaard, Copenhagen.

Ferguson, I.K. and J. Muller. 1976. The evolutionary significance of exine. Linnean Soc. Symp. No. 1. Academic Press, New York.

Feuer, S. and A.S. Tomb. 1977. Pollen morphology and detailed structure of family Compositae. Tribe Chichorieae II. Subtribe Micro seridinae. *Amer. J. Bot*., 64(27): 230-245.

Hodalova, I. and P. Martonfi. 1995. Pollen morphology in the Senecio Nemorensis group (Compositae) from the Carpathians, Comp. *Newsletter*, 26: 61-70.

Hall, M.H. 1928. *The genus Haplopappus, a phylogenetic study in the Compositae*. Carnegic Inst. Wash. Publ. No. 389.

Heslop-Harrison, I. 1969. Scanning electron microscopic observations on wall of the pollen grain of *Cosmos bipinnatus* (Compositae). *Proc. Engis Stereoscan Collog*., 9: 89-96.

Heywood, V.H., J.B. Harborne and B.L. Turner. 1977. *The Biology and Chemistry of Compositae*. Vol. 1. Academic Press, London & New York.

Huang, T. 1972. *Pollen flora of Taiwan.* National Taiwan University Botany Department Press.

Ikuse, M. 1956. *Pollen grains of Japan*. Hirokawa Publishing Co., Tokyo.

Jain, R.K. and S. Nanda. 1966. Pollen morphology of some desert plants of Pilani Rajasthan. *Palynol. Bull*., II & III: 56-69.

Keeley, S.C. and S.B. Jones. 1977. Taxonomical implications of external pollen morphology to *Vernonia* (Compositae) in the West Indies. *Amer. J. Bot*., 64: 576-584.

Kremp, G.O.W. 1965. *Encyclopaedia of pollen morphology*. Univ. Ariona Press, Tuscon, U.S.A.

Malik, N.A., S.A. Rehman and A.J. Rahmad. 1964. Pollen morphology of some Pakistani Medicinal Plants. *Pak. J. Sci. Ind. Res*., 7: 130-136.

Manten, A.A. 1970. Ultra-violet and electron microscopy and their applications in palynology. *Rev. Palaeobot. Palynol*., 10: 5-37.

Mascarenhas, J.P. 1975. The biochemistry of angiosperm pollen development. *Bot. Rev*., 41: 259-314.

Meo, A.A., H.M.I. Hafiz, F. Baig and N.A. Baig. 1988a. Studies of pollen morphology of some Gramineous (Poaceae) species. *Sarhad J. of Agric*., 4: 99-103.

Meo, A.A., H.M.I. Hafiz, F. Baig and N.A. Baig. 1988b. Pollen morphology and systematic relationships among *Graminaceous* (Poaceae) species. *J. Pure & Applied Sci*., 7(2): 15-20.

Meo, A.A., H.M.I., Hafiz, F. Baig. 1989. Pollen grain description of 13-non-allergic and 4-allergic Graminaeceous (Poaceae) species. *J. Pure and Applied Sci*., 8(2): 19-26.

Meo, A.A. 1999. Impact of pollen and inter-genetic crosses between Graminaceous (Poaceae) plants. *Pak. J. Biological Sci*., 2(3): 809-812.

Mumtaz, A.S. and M.A. Khan and T. Akhtar. 2000. Palynological studies of *Artemisia* L., from Murree and Hazara. *Pak. J. Forestry*, 50(1-2): 57-65.

Nair, P.K.K. 1965. *Pollen grains of Western Himalayan Plants*, Asia Publishing House, Bombay.

Nair, P.K.K. 1972a. Pollen-spore morphology and plant phylogeny. *Palaeoecology of Africa, the surrounding Island & Antartica*, VII: 5-6.

Nair, P.K.K. 1972b. Pollen evolution in relation to floral evolution with particular reference to Sympetalae II. *J. Palynol*., 8: 105-122.

Nair, P.K.K. and R. Lawrence. 1985. *Pollen morphology and Indian Compositae*. Scholar Publishing House, Lucknow, India.

Nakajima, J.N. and R. Monteiro. 1995. Uniformidade polinica em 18 species Brasileiras De Stevia Cav. (Eupatorieae-Asteraceae). *Naturalia, Sao Paulo*, 20: 9-19.

Nasreen, U. and M.A. Khan. 1998. Palynological studies of *Matricaria chamomilla* L., (Babuna) and its related genera. *Hamdard*., 4: 94-97.

Perveen, A. and M. Qaiser. 2002. Pollen Flora of Pakistan – XVIII. Amaranthaceae. *Pak. J. Bot*., 34(5): 375-383.

Pinar, N.M. and E.O. Dönmer. 2000. Pollen morphology of some Turkish endemic Helichysum Gaertner species (Compositae). *Pak. J. Bot*., 32(2): 295-301.

Punt, W., S. Blackmore, S. Nilsson and A. le Thomas. 1994. *Glossary of Pollen and Spore Teminology*. LPP foundation, Utrecht, LPP contributions Series No. 1.

Reitsma, T. 1970. Size modification of recent pollen grains under different treatment. *Rev. Palaeobot. Palynol*., 69: 23-47.

Rao, A.R. and P. Shukla. 1975. *Pollen Flora of Upper Gangetic Plain*. Today & Tomorrow's Printers and Publishers, New Delhi.

Sachdeva, S.K. and C.P. Malik. 1986. *Experimental plant taxonomy*. Kalyani Publications, New Delhi.

Saddiqa, A. 1964. Pollen grains of Medical plants. *Pak. J. Sci. Ind. Res*., 7: 130.

Selling, O. 1948. *Hawaiian Pollen Statistics*. Bishop Museum Publ. Hanolula.

Skvarla, J.J., V.C. Pastel and A.S. Tomb. 1977. Pollen morphology in the Compositae and in related families. In: Biology and Chemistry of the Compositae. (Eds.): V.H. Heywood, J.B. Harborne, H.L. Turner. Academic Press, London.

Serivastava, V. 1976. Pollen morphology of *Tagetes* Linn. *Palynol*., 12: 143-147.

Stanley, R.G. and H.F. Linskens. 1974. *Pollen*. Springer-Verlag. New York.

Stewart, R.R. 1977. *An annotated catalogue of vascular plants of West Pakistan*, Fakhri Printing Press, Karachi.

Tomb, S.A., D.A. Larson and J.J. Skvarla. 1974. Pollen morphology and detailed structure of family Compositae tribe Cichoriea I. Subtribe Stephanomesiinae. *Amer. J. Bot*., 61: 486-498.

Tomsovic, P. 1997. Some Palynological observations on the genus *Echinops* (*Asteraceae*) and their taxonomic implications. *Preslia, Praha*, 69: 31-33.

Vincent, P.L.D. and G.F.M. Norris. 1989. An SEM study of the external pollen morphology in *Senecio* and some related genera in the subtribe Senecioninae (Asteraceae-Senecioneae). *S. Afr. Tydskr. Plantk*, 55: 304-309.

Walker, J.W. 1975. The bases of Angiosperm phylogeny; Palynology. *Ann. Miss. Bot. Gard*., 62: 664-723.

Walker, J.W. 1976. Evolutionary significance of exine in pollen of primitive Angiospeams. In: *The evolutionary significance of the exine*. (Eds.): I.K. Feruson and J. Muller. Academic Press, London, pp. 251-308.

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

Wodehouse, R.P. 1935. *Pollen grains*. McGraw Hill. New York.

Zahur, M.S., A.A. Bhutta and M. Ashraf. 1975-78. Palynological studes of the a) plants growing in Punjab, b) seasonal variation in the frequencies of airborne pollen spores which cause allergies and asthma with species reference to "Central Punjab" Pakistan Science Foundation (PSF). Final Research Report.

(Received for publication 15 June 2003)