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Full Length Research Paper

Palynological studies in tribe Chlorideae (Poaceae) from salt range of Pakistan

Farooq Ahmad[1*], Mir Ajab Khan[2], Mushtaq Ahmad[2], Muhammad Zafar[2], Ameer Khan[3] and Zafar Iqbal[4]

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan.
[2]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
[3]Department of Biological Sciences, University of Sargodha, Pakistan.
[4]Department of Botany, Hazara University, Mansehra, Pakistan

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Pollen morphology of five species belonging to three genera of tribe Chlorideae (Poaceae) was examined by light microscopy (LM) and scanning electron microscopy. The studies showed that pollen in all species were circular in polar view, however, there are variations in equatorial view of pollen and other quantitative characters that is, polar and equatorial diameter, pore diameter and exine thickness, that are valuable in the identification and differentiation of species. Average pollen fertility in the tribe is 77.37%. Verrucate type of sculpturing is found in all species except *Tetrapogon villosus*, which showed the rugulate type of sculpturing and can be differentiated from *Tetrapogon cenchriformis* on the basis of its sculpturing pattern. The studies revealed that pollen characters are important in the taxonomy of grasses at the specific and generic level and can be useful in delimiting taxa of different tribes.

Key words: Palynological studies, chlorideae, salt range.

INTRODUCTION

Poaceae is one of the largest families among the angiosperms and is represented in every phytogeographic region in the world, comprising about 10,000 species and 651 genera (Clayton and Renvoize, 1986). The tribe Chlorideae belongs to sub family Chloridoideae, having about 45 genera throughout the tropics in the world; out of these, 7 genera and 15 species are present in Pakistan. In this study, from salt range area, 5 species belonging to 3 genera of this tribe are collected. Two problematic genera *Chloris* and *Tetrapogon* have two species each, while *Cynodon* has one species. *Tetrapogon cenchriformis* is collected from the area and it is the new report from Pakistan and not previously mentioned in the flora of Pakistan.

Pollen morphology has proved to be a valuable tool in plant taxonomy. Pollen morphology was not considered in the earlier taxonomic studies. Palynology can be helpful in solving problems related to grass systematics and can provide basis for additional features for identification of plant species (Aftab and Parveen, 2006). Pollen morphology of grasses has been studied by Kohler and Lange (1979), Chaturvedi et al. (1994, 1998) and Ma et al. (2001).

In this study, both qualitative and quantitative characters of pollen in problematic species of tribe Chlorideae were studied, to identify and differentiate species at the specific and generic level, as some characters such as grain size and sculpturing pattern are of significance in taxonomy of grasses (Woodehouse, 1935).

MATERIALS AND METHODS

The research work was conducted in the Experimental Taxonomy lab and Herbarium of Quaid-i-Azam University, Islamabad. The research work is confined to palynological studies of 5 species belonging to 3 different genera of tribe Chlorideae collected from salt range of Pakistan.

Light microscopy (LM) and scanning electron microscopy (SEM) was used to study pollen morphology and the terminology used is that of Erdtman (1952) and Moore and Webb (1978).

Florets were dissected and anthers were placed on the slide with the help of forceps, added a drop of 45% acetic acid and crushed with iron rod. Pollens were acetolysed according to modified method of Ahmad et al. (2008), who followed Erdtman (1952).

*Corresponding author. E-mail: farooq_qau76@yahoo.com. Tel: +92-306-5118498.

Stirred with needle for equal distribution of pollens, placed the cover slip and sealed the slide edges by transparent nail polish. Slides were labeled with their name, locality and voucher number. The slides were kept in wooden slide cases in vertical position.

The following pollen parameters were studied under light microscope for pollen morphology; shape in polar and equatorial view, polar diameter, equatorial diameter, P/E ratio, number of pores, pore diameter and exine thickness.

For pollen studied (sculpturing pattern) by SEM (scanning electron microscopy), anthers were crushed in 45% acetic acid and one to two drops of material containing acetolysed pollen were mounted on metallic stubs with a fine pipette and coated with gold in vacuum coater and examined with a Jeol microscope (JSM 1200).

To determine pollen fertility, acetocarmine and glycerin jelly was used by the modified techniques used by Khan and Stace (1999). Anthers were squashed in a drop of acetocarmine. Debris was removed gently and cover slip was placed on it. The slides were observed at low magnification (10 x). The number of stained and unstained pollen was counted. Fully stained pollen was considered fertile, while unstained and deformed pollen were considered unfertile.

RESULTS

The qualitative and quantitative characters of pollen of 5 species belonging to tribe Chlorideae are given as follows:

Chloris barbata Sw.

The pollen is circular in polar view and spheroidal to oblate spheroidal in equatorial view. The polar axis diameter is 23.50 µm (22 to 25.90 µm) and equatorial axis diameter is 24.37 µm (17.5 to 27.5 µm). P/E ratio is 0.95. Pollen is monoporate and ectoporate. Pore diameter is 1.40 µm (1 to 2 µm) and exine thickness is 0.75 µm (0.65 to 0.95 µm). Pollen fertility is 53.84%. Sculpturing is verrucate and verrucae are narrowly spaced (Figure 1a).

Chloris dolicostachya Lag.

The pollen is circular in polar view and spheroidal to oblate spheroidal in equatorial view; the polar axis diameter is 21.89 µm (20 to 25 µm) and equatorial axis diameter is 23.18 µm (20 to 27.5 µm). The P/E ratio is 0.94 while the pollen is monoporate and ectoporate. The pore diameter is 1.5 µm (1.0 to 2.0 µm) and exine thickness is 0.89 µm (0.75 to 1.0 µm). Pollen fertility is 84.16%. Sculpturing is verrucate (Figure 1b).

Cynodon dactylon (Linn.)Pers

The pollen is circular in polar view and spheroidal to sub prolate in equatorial view. The polar axis diameter is 22.5 µm (15 to 22.5 µm) and equatorial axis diameter is 19.54 µm (15 to 22.5 µm). The P/E ratio is 1.15 and pollen is monoporate and ectoporate. Pore diameter is 1.65 µm (1.25 to 2.0 µm) while the pollen fertility is 79.68%.

Sculpturing is verrucate and verrucae are narrowly spaced (Figure 1c).

Tetrapogon cenchriformis (A. Rich.)Clayton

The pollen is circular in polar view and prolate spheroidal in equatorial view. The polar axis diameter is 24.37 µm (20 to 30 µm) and equatorial axis diameter is 23.5 µm (20 to 27.5 µm). P/E ratio is 1.03. The pollen is exoporate and monoporae. Pore diameter is 2.7 µm (2.5 to 3.5 µm) and exine thickness is 0.95 µm (0.75 to 1.00 µm). Pollen fertility is 89.18%. Sculpturing is verrucate and verrucae are narrowly spaced (Figure 1d).

Tetrapogon villosus Desf.

The pollen is circular in polar view and oblate spheroidal in equatorial view. Polar axis diameter is 24.16 µm (22.5 to 27.5 µm) and equatorial axis diameter is 24.77 µm (22.5 to 30 µm). P/E ratio is 0.97. The pollen are ectoporate and monoporate. The pore diameter is 1.5 µm (1.2 to 1.7 µm) and exine thickness is 0.93 µm (0.75 to 1.0 µm). Pollen fertility is 80%. Sculpturing is rugulate and the rugulae are widely spaced (Figure 1e).

DISCUSSION

Few studies have been conducted on the pollen morphology of Chloridoideae (Huang, 1975). According to Liu et al. (2005), pollen grains in subfamily Chloridoideae are generally radically symmetrical and prolate spheroidal. In this study, all the species in tribe Chlorideae showed circular pollen in polar view. Circular nature of pollen is the structural adaptation of grasses for effective pollination by insects (Gimenis, 1991; Edeoga and Okoli, 1996). Pollen in *C. barbata* are larger than *C. dolicostachya*, having polar diameter (23.50 µm) and equatorial diameter (24.37 µm), while pore diameter (1.5 µm) and exine thickness is more in *C. dolicostachya* than *C. barbata*. Pollen in both species of *Tetrapogon* differs with each other in equatorial view, as it is oblate spheroidal in *T. villosus* and prolate spheroidal in *T. cenchriformis*. Pollen is large in polar view (24.16 µm) in *T. cenchriforms,* but small in equatorial view (23.5 µm) than *T. villosus*. Other quantitative characters such as P/E ratio pore diameter and exine thickness are recorded more in *T.cenchriformis*. These variations in size pore diameter and exine thickness serve as point of differentiation in different species (Mbagwu et al., 2008).

Siddiqui and Qaisar (1988) studied 4 species of Chlorideae for palynology and average grain size was

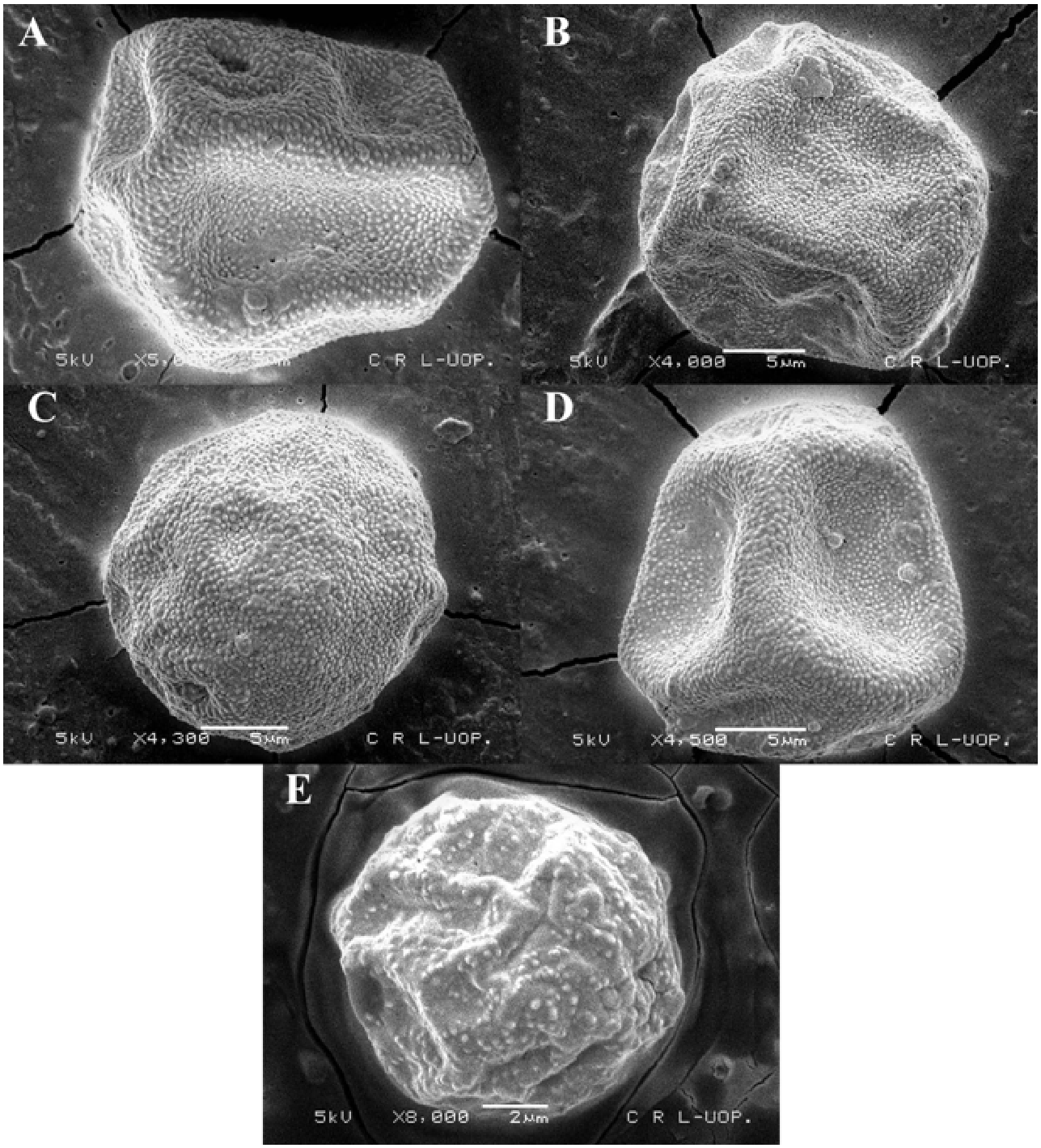

Figure 1. A, Pollen of *C. barbata* showing verrucate sculpturing; B, *Chloris dolicostachya* pollen with verrucate sculpturing; C, *C. dactylon* pollen with verrucate sculpturing; D, pollen of *T. cenchriformis* showing verrucate sculpturing; E, pollen of *T. villosus* showing rugulate sculpturing.

recorded, 18.59 to 31.46 µm, while in this study, pollen size ranged from 15 to 30 µm. In *C. dactylon*, pollen are spheroidal to sub prolate in equatorial view and pore diameter (1.65 µm) in this species more than the otherspecies studied in the tribe except *T. cenchriformis* which has maximum pore diameter and grain size than other species. Siddiqui and Qaisar (1988) found the grain size in *C. dactylon* (35.14 µm), but in this study pollen diameter in this species is 15 to 22.5 µm. Parveen (2006) studied *Cynodon arculatus*, having pollen diameter from, 19.74 to 24.71 µm. According to her observations, pollen are spheroidal and monoporate. Pollen is also

Table 1. Pollen characters of different species in tribe Chlorideae.

Species name with voucher number	Shape in polar view	Shape in equatorial view	Sculpturing pattern	Polar axis diameter (µm)	Equatorial axis diameter (µm)	P/E ratio (µm)	Pore diameter (µm)	Exine thickness (µm)	Pollen fertility (%)
Chloris barbata (369)	Circular	Spheroidal to oblate spheroidal	Verrucate	23.50 (22-25.90)	24.37 (17.5-27.5)	0.95	1.40 (1.0 -2.0)	0.75 (0.65 -0.95)	53.84
Chloris dolicostachya (330)	Circular	Spheroidal to oblate spheroidal	Verrucate	21.89 (20-25)	23.18 (20-27.5)	0.94	1.5 (1.0-2.0)	0.89 (0.75-1.0)	84.16
Cynodon dactylon (17)	Circular	Spheroidal to sub prolate	Verrucate	18.5(15-22.5)	19.54 (15-22.5)	1.15	1.65 (1.25-2.0)	0.75 (0.6-1.0)	79.68
Tetrapogon cenchriformis (268)	Circular	Prolate spheroidal	Verrucate	24.37 (20-30)	23.5 (20-27.5)	1.03	2.7 (2.5-3.5)	O.95 (0.75-1.0)	89.18
Tetrapogon villosus(32)	Circular	Oblate spheroidal	Rugulate	24.16 (22.5-27.5)	24.77 (22.5-30)	0.97	1.5 (1.2-1.7)	0.93 (0.75-1.0)	80

monoporate and ectoporate in this study, as the number and position of aperture is of prime significance in palynology (Ogbebor, 1996). Variations are found in equatorial view as spheroidal to oblate spheroidal pollen are found in different species, even differences are found in two species of same genus that is, *Tetrapogon*, but in genus *Chloris* both species are similar in equatorial view. So these variations observed in qualitative as well as qualitative characters may be helpful in the identification of different species and genera in the tribe. All the species in this tribe showed the verrucate type of sculpturing except *T. villosus* which has regulated type of sculpturing; hence, sculpturing pattern is an important tool to differentiate *T. villosus* from other species of the genus and tribe.

Pollen fertility ranges from 53.84 to 89.18% (Table 1). The average pollen fertility in the tribe Chlorideae is 77.37%. Maximum pollen fertility is recorded in *T. cenchriformis*. The degree of fertility of hybrids may give some indication of the degree of relationship between its parents. In general, hybrids between species of a genus that are not closely related tend to be sterile or of low fertility, whereas hybrids between taxonomically more closely related species or intra-specific taxa tend to be more fertile (Khan, 1991).Thus, there is a correlation between hybrid fertility and taxo-nomic relationship. Pollen fertility is a valuable tool in taxonomic studies to distinguish putative hybrids from the parent plants and is also useful to determine the degree of fertility in those plants that are grown under unfavorable condition (Lawrence, 1951). The genetic variations of a flora can be observed by studying their pollen fertility (Tellaria, 1991). It is a helpful tool to assess the stability of species in a particular area.

Conclusion

This study shows that all the species present in tribe Chlorideae have circular pollen. In *Chloris* and *Tetrapogon,* variations are observed in qualitative and quantitative characters of pollen. These variations are valuable in the identification of species. Verrucate type of sculpturing pattern is observed in the tribe except *T. villosus.* Maximum pollen fertility is observed in *T.cenchriformis,* it is concluded from this study that variations exist in qualitative and quantitative characters of pollen, in different species of the tribe and sculpturing pattern in genus *Chloris* that are helpful in

identification, differentiation and delimiting of different taxa.

REFERENCES

Aftab R, Perveen A (2006). A palynological study of some cultivated trees from Karachi. Pak. J. Bot. 38(1): 15-28.

Ahmad M, Khan MA, Hussain A, Zafar M, Sultana S (2008). Chemotaxonomic standardization of herbal drugs: Milk thistle and Globe thistle. Asian J. Chem., 20(60): 1443-1453.

Chaturvedi M, Yunus D and Datta K (1994). Pollen morphology of *Sorghum* Moench- sections Eu-Sorghum and para- Sorghum Grana, 33: 117-123.

Chaturvedi M, Datta K, Nair PKK (1998). Pollen morphology of *Oryza* (Poaceae). Grana, 37: 79-86.

Clayton WD, Renvoize SA (1986). Genera Gramineum, Grasses of the World. Kew bulletin additional series 13, Royal Botanic gardens, Kew. p. 389.

Edeoga HO, Okoli BE (1996). Apomictic behaviour in Costus after *C. lucanusianus* (Costaceae) complex in Nigeria. Feddes Report, 107: 75-85.

Erdtman G (1952). Pollen morphology and plant taxonomy of Angiosperms (An introduction to Palynology 1). Almqvist and Wiksell, Stockholm. Chronica Botanica Co., Waltham, Mass ., U.S.A. p. 538.

Gimenis M (1991). Some morphological adaptations in bees (*Hymenos apoidea*) for collecting pollen from *Ludwigia elegans* (Onagraceae). Rev. Brasil. Entomol. 35: 413-422.

Huang CC (1975). Pollen grain of Formosan plants. Taiwania. 225-267.

Khan MA (1991). Cytotaxonomic studies in some species of *Brachypodium* P. Beauv. Pak. Phyton, 3: 141-151.

Khan MA, Stace CA (1999). Breeding relationships in the genus *Brachypodium* (Poaceae, Pooideae). Nord. J. Bot. Copenhagen, ISSN 0107-055 X. 19: 257-269.

Kohler E, Lange E (1979). A contribution to distinguishing cereal from wild grass pollen by LM and SEM. Grana, 18: 133 -134.

Lawrence GHM (1951).Taxonomy of vascular plants. Mc Millan Co. New York.

Liu Q, Zhao N, Hao G (2005). Pollen morphology of *Eustachys tenera* (Chloridoideae, Gramineae). Pak. J. Bot. 37(3): 503-506.

Ma GH, Zhao NX, Hu XY (2001). Pollen morphology and poly-aperture in *Paspalum*. J. Trop. Subtrop. Bot. 9: 201-204.

Mbagwu FN, Okafor VU, Okolo AE (2008). Comparative palynological characters of four species of family Poaceae. Int. Sci. res. J. 1(2): 127-129.

Moore PD, Webb JA (1978). An illustrated guide to pollen analysis. Hodder and Stoughton, London. p. 133.

Ogbebor ON (1996). Pollen morphology and plant taxonomy. An unpublished thesis submitted to Dept. of Botany, Edo State University Ekpoma, p. 21.

Parveen A (2006). A contribution to the Pollen morphology of family gramineae. World . Appl. Sci. J. 1(2): 60-65.

Siddiqui T, Qaisar M (1988). A palynological study of the family Gramineae from Karachi Pakistan. Pak. J. Bot. 20(2): 161-176.

Tellaria MC (1991). Comparative palynological analysis of species of *Medicago*L., *Melilotus* Mill and *Trifolium* L.(Leguminoseae) in the phytographic pampas region (Argentina). Industria Apicola, 1: 4-13.

Lawrence GHM (1951). Taxonomy of vascular plants. Mc Millan Co. New York.

Woodehouse RP(1935). Pollen grains, their structure, identification and significance in medicine. Hafner Publishing company, New York, p. 574.