Pak. J. Bot., 45(SI): 143-149, January, 2013.

SYSTEMATIC IDENTIFICATION OF GENUS *BRACHIARIA* ON THE BASIS OF VEGETATIVE AND FLORAL MORPHO-PALYNOLOGICAL MARKERS (LM & SEM)

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Abstract

The present study deals with the few taxonomic parameters of the 5 species of genus *Brachiaria* carried out from Pakistan as there is confusion in identification of these species particularly on the basis of morphology and palynology. In the context of morphology, characters such as spikelet differentiation, lemma and palea shape and size, spike color, shape and size, glumes length and width were found useful in the delimitation of taxa in the genus. The inflorescence morphology particularly the spikelet was examined in detail and found to be highly variable. All the available records are listed and mapped. The palynological analysis provides qualitative and quantitative systematic data. The results showed some distinct palynological features. Variations in pollen characters like apertural form, exine ornamentation, pollen shape and size, polar and equatorial diameter and P/E ratio were observed. *Brachiaria villosa* was given in the flora of Pakistan and was considered to be of doubtful origin in Pakistan by Stewart (1990). This species is also confirmed to occur in Pakistan in the present study. Also there is no published data already regarding the morpho-palynological distinctions among the species of *Brachiaria* particularly from Pakistan.

Introduction

Brachiaraia Griseb, is a genus of annual and perennial grasses belonging to the tribe Paniceae, sub-family, Panicoideae of the family Gramineae. This genus is represented by a total of about 50 species of which 17 are reported from India (Bor, 1960). Trinius (2006) first recognized *Brachiaria* as a section of *Panicum* on the basis of its recemose inflorescence. The section was elevated to generic level by Grisebach (1993). Nash (2003) in his generic treatment was the first to note that the first glume of the inclusive species was turned toward the rachis. Reinheimer *et al*., (2005) observed that 11 developmental features not discernible in mature inflorescence were found in some species of *Brachiaria*, these features are mentioned as, direction of branch differentiation, origin of Primary branches, development of the main axis, direction of spikelet, direction of glume, lemma and palea, position of lower glume, pattern of distal floral development, glume elongation, pattern of gynoecium abortion, differential pollen development and size of the floret meristem.

Morphological characters are helpful in identification and delimitation of taxa and genera into tribes and sub-families (Yousuf *et al*., 2008). These characters had been recognized as basic criteria for identification and authentication of plants (Sultana *et al*., 2011). Clayton & Renvoize (2006) recognized 6 subfamilies, Kellogg (1998) recognized 13 and the "in progress" data of the Grass Phylogenetics Working Group could be used to justify recognition of at least 16 subfamilies, often composed of one or a few species. Descriptive study of morphological characters helps to understand the evolution basis of Gramineae. Soreng & Davis, (1998) reported that in the last couple of years, a clear picture has formed of the evolutionary history of the grass family. Morphological features can also be utilized in the phylogeny of grasses. Anon., (2000) reported that the phylogeny shows that the spikelet must have originated in several steps. The earliest grasses had three stigmas, a relict of the three fused carpels that they inherited from their ancestors; this number was reduced to 2 after the speciation event. The earliest species also had, like their non-grass ancestors, 6 stamens.

Grasses are currently identified based on their floral characters, but a problem encountered with the system of identification is that grasses do not flower for a greater part of their life cycle (Gilani *et al*., 2002). Hence, a biosystematic approach could be used in tackling this problem through gathering of data from various studies such as palynology, anatomy, cytology, epidemiology etc. Palynology, the study of pollen grains, is one of the most effective tools for identification. The study of different palynological characters such as shape of the pollen, pollen class, presence or absence of the pore, position of the pore, exine and intine thickness, P/E ratio and sculpturing of the pollen surface helps us to classify and identify grasses into their various tribes and genus and thus adds to our knowledge on the biosystematics of grass species. Palynological studies have been used successfully to clarify taxonomic status and also help in the identification of species (Chaudhary *et al*., 2001). As Binzet (2011) found the taxonomic effectiveness of pollen type at specific level. Moreover Perveen & Qaiser (2012) highlighted the significance of these palynological characters in grasses systematic as well.

The present studies give the complete and comprehensive vegetative and floral morphological description and compare/determine the intraspecific relationship and patterns of variation associated with the morphological and palynological features/characteristics among the taxa studied of genus *Brachiaria* present in Pakistan.

Material and Methods

Morphological studies: Five specimens of the genus *Brachiaria* were studied under the binocular light microscope. Measurements of various parts of a specimen were taken 3-5 times to ensure the reading and calculated its mean. The following morphological characters of the specimen were observed: Height of the plant, number of culms, number of nodes, length and width of leaf sheath, blade, panicle, spike, glume, lemma, palea and length of stamens, color of leaf sheath, pubescence of leaf sheath, blade, rachis, glume, lemma, palea, shape of glume, lemma and palea, texture of rachis, number of stamens and carpels. Characteristics were reconfirmed by using various floras, Nasir & Ali (1982), Tutin & Heywood (1972) and Saldanha & Nicolson (1976).

Palynological studies: Palynomorph study was done according to the modified method of Wodehouse technique (Ronald, 2000). Pollens were acetolized and stained by using glycerin jelly. Glycerin jelly was prepared according to modified method (Ahmad *et al*., 2003). The pollen fertility estimation was carried out by employing the techniques used by Meo & Khan (2006). Microphpotographs of pollen samples were taken by Nikon (FX-35) Camera equipped light microscope and Scanning Electron Microscope. The method for SEM analysis was followed by the Terrel & Wergin (1979) and Hilu & Wright (1984).

Qualitative characters studied under light microscope for pollen morphology were type of pollen, shape in polar & equatorial view, presence or absence of pore, colpi and spines, shape of pore (ora), presence of cavities and patches and sculpturing. The quantitative characters were polar & equatorial diameter, number of pores, exine thickness, intine thickness and pollen fertility.

Statistical analysis: Data was evaluated by co-relation matrix and cluster analysis to determine .inter specific relationship. Dendrogram was constructed on the basis of unweight pair group method with arithmetic average (UPGMA).The computer software SPSS.V 11.0 was use for this purpose.

Results and Discussion

***Brachiaria distachya* L.**

Morphology	Creeping annual, Culms, 9-74cm tall, ascending from a prostrate base. Leaf blades, linear to narrowly lanceolate, 2.7-12.5cm long and 0.3-1.2mm wide, leaf sheaths glabrous to sparsely pilase, ligule a fringe of hairs, 0.5-1mm long. Inflorescence, racemes with the bristles subtending the spikelets. Spikes/florets, spikes greenish, 3.1-10.9cm long, borne along a central axis, subtended by the bristles, bristles 0.7-1.3cm long, light greenish, rachis narrowly winged, spikelets elliptic, 1.5-2mm long, glabrous, acute, glumes dissimilar, reaching apex of florets, thinner than fertile lemma, lower glume ovate, lower glume apex obtuse, 5-nerved, upper glume oblong, upper glume apex acute, 5-7-nerved, lemma elliptic, lemma surface rugulose, lemma margins involute, lemma apex acute, 5-nerved, 0.2-0.4 mm long and mostly 0.1mm wide, palea involute, indurate, 0.3mm long and 0.1mm wide, anthers 3, Graminoid life form. (Table 1).
Palynology (LM & SEM)	In equatorial view, the pollens are circular and semi-circular (Fig. 1a). In polar view, the pollen are semi-angular, prolate and spheroidal (Fig. 1b). Polar diameter is 24.5µm (24-25µm) and equatorial diameter is 22.5µm (20-25µm). P/E ratio is 1.08 and exine thickness is 0.9µm (0.9-1µm) and intine thickness is 1.1 (0.8-1.4). Apertural form of pollen is monocolpate type and position is proximal. Tectum is scabrate. Pore is endoporus. Percentage of pollen fertility in this species is 78.66% (Fig. 6).

***Brachiaria eruciformis* (Sm.) Griseb.**

Morphology	Loosely tufted annual, Culms, loosely tufted, slender, much branched, geniculately ascending nodes, softly hairy, 4.9-37cm tall. Leaf blades, linear to lanceolate, glabrous or pilose, 2.9-15.7cm long and 0.2-1.5mm wide, leaf sheaths glabrous or loosely tuberculae-hairy, ligule ciliate, about 0.2-1mm. Inflorescence, racemes with bristles, subtending the spikelets. Spikes/florets, spikes greenish, 2-10.2 cm long, borne along a central axis, subtended by the bristles, bristles light greenish, 0.5-1.2 cm long, rachis triquetrous, narrow, ciliate or scabrous, spikelets elliptic, 1.7-2.5mm long, a little hairy, spikelets single, sub-acute, lower glume ovate, lower glume apex truncate, nerveless, lower glume surface glabrous or pubescent, membranous, upper glume oblong, upper glume apex acute, 5-nerved, lemma elliptic, lemma surface smooth, lemma margins involute, lemma apex obtuse, 5-nerved, glossy, 0.3-0.4 mm long and 0.1mm-0.2mm wide, hard palea, mostly 0.2mm long and 0.1mm wide, anthers 3, Stoloniferous annual. (Table 1).
Palynology (LM & SEM)	In equatorial view the pollen are semi-angular and angular while in polar view they are circular to semicircular. (Figs. 2a, 2b). Polar diameter is 23µm (21-25µm) and equatorial diameter is 22.5µm (20-25 µm). P/E ratio is 1.02 and exine thickness is 0.9µm (0.9-1µm) and intine thickness is 1.3 (1.1-1.5).

Apertural form of pollen is monocolpate type and pollen is monoaperturates. Tectum is scabrate. (Table 1). Pore is endoporus (Fig. 2b). Pollen cavities are present. Percentage of pollen fertility in this species is 94.85% (Fig. 6).

***Brachiaria ramosa* L.**

Morphology: Culms, may grow erect or prostrate along the ground, ascending, 37.9-59cm tall, nodes along the stem are minutely to shortly hairy. Leaf blades, narrowly lanceolate, 3.1-15.7cm long and 0.2-1.5mm wide, margins thickened and slightly wavy, apex acuminate, leaf sheaths glabrous to pubescent, ligule short, ciliate, 1-2mm long. Inflorescence, racemes with bristles subtending the spikelets. Spikes/florets, spikes greenish, 2.8-10.2cm long, borne along a central axis, subtended by the bristles, bristles light greenish, 0.6-1.2cm long, minute hairs at rachilla, rachis triquetrous, hispid, spikelets elliptic, 2-3.5mm long, little hairy spikelet, glabrous, acute, lower glume broadly ovate, lower glume apex acute or obtuse, 5-nerved, upper glume ovate, upper glume apex acute, 5-7 nerved, lemma ovate, lemma surface smooth, lemma margins involute, lemma apex acute, papery lemma, 5-7 nerved, 0.3mm long and 0.1mm wide, hard palea, mostly 0.2mm long and 0.1mm wide, anthers 3, Adventitious (fibrous) roots. (Table 1).

Palynology (LM & SEM): In equatorial view the pollen are circular while in polar view they are circular, semicircular and tubular. (Figs. 3a & 3b). Polar diameter is 32.5µm (25-40µm) and equatorial diameter is 30µm (25-30µm). P/E ratio is 1.08 and exine thickness is 0.85µm (0.7-1µm) and intine thickness is 1.15 (1.0-1.3). The position of pore is proximal and pollen is monoporate. Tectum is scabrate (Fig. 6). Pore is endoporus. Percentage of pollen fertility in this species is 61.42% (Table 2).

***Brachiaria reptans* L.**

Morphology: An annual grass, Culms, decumbent, rooting at nodes, 11.2-57.6cm tall. Leaf blades, lanceolate, 1.1-17.2cm long and 0.1-0.5mm wide, leaf sheaths glabrous or pubescent, ligule a hairy fringe, 1-1.5 mm long. Inflorescence, racemes with the bristles subtending the spikelets. Spikes/florets, spikes greenish, 1.1-8.6cm long, borne along a central axis, subtended by the bristles, bristles greenish, 0.6-0.9cm long, rachis triquetrous, spikelets ovate to broadly elliptic, 1-2mm long, glabrous, acute, lower glume orbicular, lower glume apex truncate, nerveless, upper glume ovate, upper glume apex acute, 7-nerved, lemma elliptic, lemma surface rugose, lemma margins involute, lemma apex sub-acute, 5-nerved, 0.4 mm long and 0.2mm wide, palea a hard structure, 0.3mm long and 0.1mm wide, anthers 3 (Table 1).

Palynology (LM & SEM): In equatorial view the pollen are sub-prolate, prolate and spheroidal while in polar view they are circular. Polar diameter is 30.5 µm (25-35 µm) and equatorial diameter is 30 µm (25-35 µm). P/E ratio is 1.0 and exine thickness is 0.15 µm (0.1-0.4 µm) and intine thickness is 1.05 (0.9-1.2). Apertural form of pollen is monocolpate type. Tectum is scabrate. (Table 1). Pore is endoporus. Patches on pollen surface are present. (Fig. 4a, 4b). Percentage of pollen fertility in this species is 99.22% (Fig. 6).

***Brachiaria villosa* L. (Lam.)**

Morphology: Culms, loosely tufted, slender, broadly lanceolate, 10-40cm tall. Leaf blades, broadly lanceolate, 5-6.3cm long and 0.3-1mm wide, both surfaces glabrous to densely pubescent, base rounded or sub-cordate, margins scabrous, apex acute, sheaths glabrous or pubescent, ligule ciliate, 0.5-1mm long. Inflorescence, racemes with bristles subtending the spikelets. Spikes/florets, spikes greenish, 4-7cm long, borne along a central axis, subtended by the bristles, bristles greenish, 0.6-0.8cm long, rachis triquetrous, spikelets elliptic, 2-2.5mm long, glabrous or pubescent, mostly single, acute or sub-acute, lower glume ovate, lower glume apex acute, 3-nerved, upper glume oblong, upper glume apex acute, 5-nerved, lemma elliptic, lemma surface rugulose or striate, lemma margins involute, lemma apex acute to minutely mucronate, 5-nerved, 0.4-0.5mm long and 0.3mm wide, palea 0.3mm long and 0.1mm wide, anthers 3 (Table 1).

Palynology (LM & SEM): In equatorial view the pollen are circular, semi-circular and sub-prolate while in polar view they are circular. Polar diameter is 33µm (31-35µm) and equatorial diameter is 30µm (25-35µm). P/E ratio is 1.1 and exine thickness is 0.35µm (0.3-0.5µm) and intine thickness is 1.3 (1.1-1.5). Surface of pollen is bacculate. (Fig. 5a). Pore is ectoporus (Fig. 5b). Percentage of pollen fertility in this species is 79.83% (Table 2).

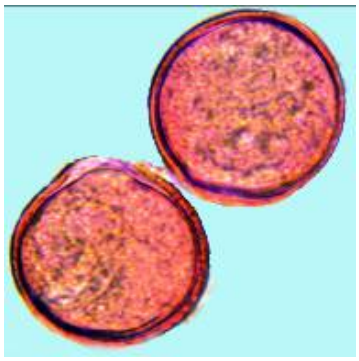

Fig. 1a. *Brachiaria distachya* (LM).

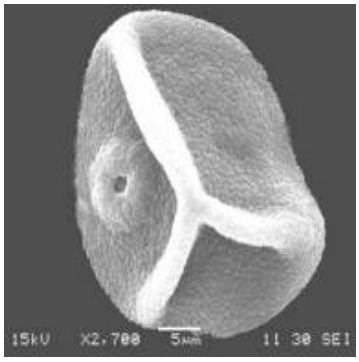

Fig. 1b. Semi-angular shaped (SEM).

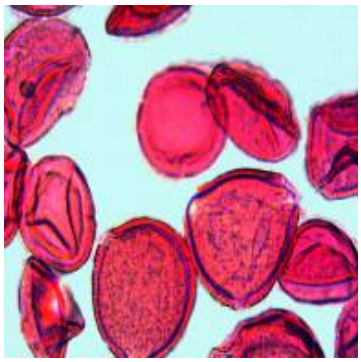

Fig. 2a. *Brachiaria eruciformis* (LM).

Fig. 2b. Endoporus pollen (SEM).

Morphology: Mostly the classification within the family Poaceae is based upon the characteristics of spikelets and their arrangements. The spikelets are a specialized condensed inflorescence unit represented in its various forms and modifications within the family Gramineae. It is generally composed of two to many (rarely single) florets. The various modifications of this basic unit lead to the diversity of the spikelets characteristics. The florets number varies from indefinite to a few or even one per spikelet.

The vegetative and floral morphology of the taxa showed that the inflorescence type was helpful in identification of grasses generally (Gilani *et al*., 2003 & 2003a). The characters of inflorescence types have been mainly utilized by Ali & Qaiser (1986) for the classification of, Grasses of Saudi Arabia. Minute microscopic characters like shape, size, pubescence and presence/absence on grooves on glumes, lemma and palea are important for the identification of the species of the genus.

Most of the *Brachiaria* species have hard palea whereas the length and width of palea varies in different species. In *B. reptans* palea is mostly 0.3 mm long whereas Tzvelev (2004) mentioned that in the genus *Brachiaria* observed lemma was almost similar to the upper glume, but often three veined and palea of the sterile flower was almost membranous. A cluster of hairs is present in almost all the species of the genus. Spikelets in each species are enclosed by involucres, composed of whorls of bristles. The size and nature of the bristles vary in different species. The length, width and color of spikes is also different in different species e.g., straw color, purple color, greenish color, light brown to blackish brown etc.

Morphologically, culm and leaf blades length is also variable in different taxa. Moreover, in a species, the culm and leaf blades length is also variable. Hooker, (1997) reported that there was variation observed in the length and width of the leaf blades of the species in the genus.

So morphologically all the species in genus show great variation not only in the different species, but also within the same species. Present study of detailed morphological characters also present the comprehensive description of *Brachiaria villosa*, which was given in the flora of Pakistan (1990), but was considered to be of doubtful origin in Pakistan by Stewart.

Palynology: The pollen grains of all the genera are radially symmetrical, isopolar and isodiametric. The pollen size is quite variable among the species indicating that pollen morphological characters are helpful at the specific level also. The size of pollen grains in polar view ranged from 23 (21-25) µm to 33 (31-35) µm. *B. eruciformis* appears to be the smallest in size whereas *B. villosa* is the largest. In equatorial view, the size range from 20 (25-35) µm to 30 (25-35) µm. *B. villosa* appears to be the smallest in size whereas *B. ramosa* and *B. reptans* are the largest (Table 2). Zahur *et al*., (1975-1978) concluded that the pollen grains in *Poaceae* are usually monoporate, annulate, almost spherical, psilate and operculate with layers about 1 µm thick.

P/E ratio ranged from 1.0 to 1.1 µm among the species. *B. reptans* appears to be the lowest while *B. villosa* comprises the highest value. Exine thickness ranged from 0.15 to 0.9 µm among the species. *B. reptans* shows the lowest value whereas *B. distachya* and *B. eruciformis* shows the highest value (Table 2). A lot of variation is also observed in the shapes of the species. In *B. distachya*, the pollen grains are semi-angular, prolate or spheroidal in polar view (Fig. 1a) whereas in *B. ramosa* the pollen grains are circular. There is no variation in the pore position in this genus. Pore is endoporus in all *Brachiaria* species (Figs. 2b, 3b) except in *Brachiaria villosa*. It is concluded that pollen size, exine thickness and shape proved to be useful features of the specific level.

Pollen fertility is useful tool to determine genetical variation. By using this aspect, we recognize the hybrids within the population and the relationship of different species. On the basis of pollen fertility, the taxonomists may recognize between intergenetic, interspecific and intraspecific hybrids. In the genus *Brachiaria*, the highest value of pollen fertility is found in *B. eruciformis* as 94.85% and the lowest value is in *B. ramosa* as 61.42% (Fig. 6). Lawrence (2009) observed that the pollen fertility is valuable for the taxonomists in attempting to distinguish putative hybrids from the parent plants and is also useful to determine the degree of fertility/stainability in those plants that are grown under unfavorable conditions.

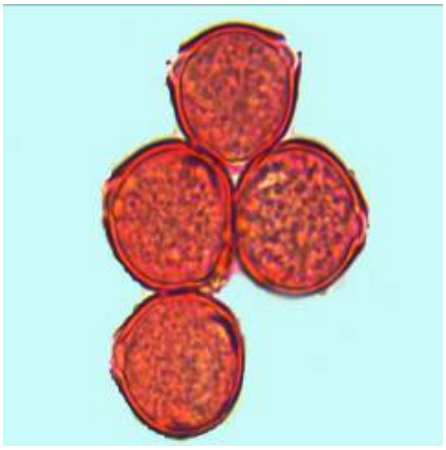

Fig. 3a. *Brachiaria ramosa* (LM).

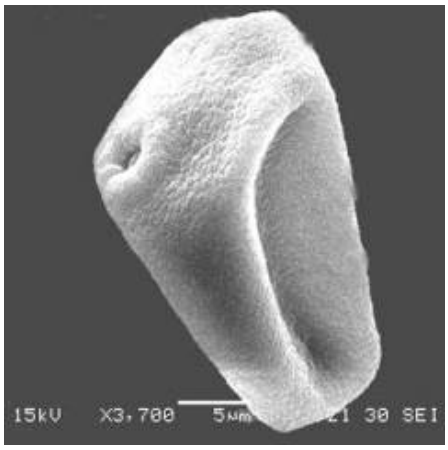

Fig. 3b. Tubular shaped pollen (SEM).

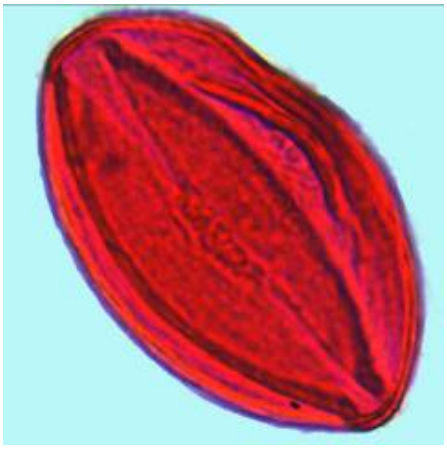

Fig. 4a. *Brachiaria reptans* (LM).

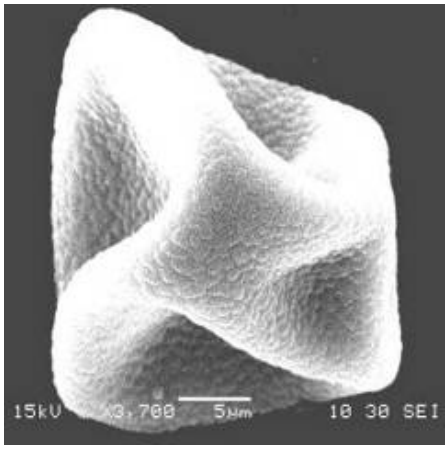

Fig. 4b. Pollen patches (SEM).

Fig. 5a. *Brachiaria villosa* (LM).

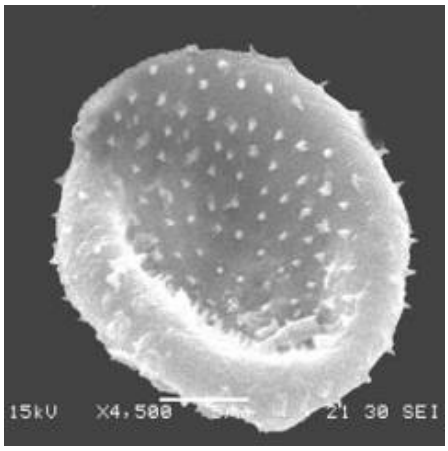

Fig. 5b. Bacculate surface of pollen.

Statistical analysis: Cluster analysis produces a hierarchical classification of taxa based on the similarity matrix. It is observed that there is highly significant correlation between the polar diameter, equatorial diameter, P/E ratio, exine thickness, intine thickness and pollen fertility of the two species *Brachiaria eruciformis* and *Brachiaria reptans*, whereas *B. distachya, B. ramosa* and *B. villosa* correlates with each other on the basis of palynological characters. These parameters are good taxonomic tools for the identification and classification of the species studied. This analysis also proved that palynological studies are taxonomically significant to delimit taxa.

Table 1. Morphological distinctions among the species of genus *Brachiaria*

Characteristics	B. distachya	*B. eruciformis*	*B. ramose*	*B. reptans*	*B. villosa*
Culms	Ascending from a prostrate base	Loosely tufted, slender	Erect or prostrate along the ground	Decumbent	Loosely tufted, slender
Leaf blades	Linear to narrowly lanceolate	Linear to lanceolate	Narrowly lanceolate	Lanceolate	Broadly lanceolate
Leaf sheaths	Glabrous to sparsely pilase	Glabrous or loosely tuberculae-hairy	Glabrous to pubescent	Glabrous	Glabrous or pubescent
Spikelets	Elliptic, acute	Elliptic, sub-acute	Elliptic, acute	Ovate to broadly elliptic, acute	Elliptic, acute or subacute
Lower glume	Ovate	Ovate	Ovate	Orbicular	Ovate
Lower glume apex	Obtuse	Truncate	Acute or obtuse	Truncate	Acute
Upper glume	Oblong	Oblong	Ovate	Ovate	Oblong
Glumes nerves	5-7 nerved	5-nerved	5-7 nerved	7-nerved	3-5 nerved
Lemma	Elliptic	Elliptic	Ovate	Elliptic	Elliptic
Lemma apex	Acute	Obtuse	Acute	Sub-acute	Acute
Lemma surface	Rugulose	Smooth	Smooth	Rugose	Rugulose or striate
Lemma nerves	5-nerved	5-nerved	5-7 nerved	5-nerved	5-nerved

Key to different species of *Brachiaria* (Trin) Griesb., on the basis of morphological distinctions

1. + Rachis of racemes flat, ribbon like, narrowly winged
   Rachis of racemes solid, triquetrous or cresenttic, sometime with very narrow wings
2. + Upper lemma smooth, shining, obtuse …….. **2. *B. eruciformis***
   Upper lemma granulose to rugose, subacute to mucronulate
3. + Spikelets 1.5-2.2 mm long, without a stipe; lower glume ¼ the length of the spikelet, rarely a little longer …… ……..… **3. *B. reptans***
   Lower glume a third to half the length of the spikelet, rarely shorter ……..… **4**
4. + Upper glume and lower lemma membranous, glabrous or pubescent …….……. **5. *B. ramosa***
5. + Upper lemma transversely rugulose
   Lower glume usually grooved ..…….. **6. *B. villosa***

Table 2. Palynological variations among the genus *Brachiaria*

Characteristics	*B. distachya*	*B. eruciformis*	*B. ramose*	*B. reptans*	*B. villosa*
Polar diameter	24.5 (24-25)	23 (21-25)	32.5 (25-40)	30 (25-35)	33 (31-35)
Equatorial diameter	22.5 (20-25)	22.5 (20-25)	30 (25-35)	30 (25-35)	20 (25-35)
P/E ratio	1.08	1.02	1.08	1.0	1.1
Exine thickness	0.9 (0.9-1)	0.9 (0.9-1)	0.85 (0.7-1)	0.15(0.1-0.4)	0.35 (0.3-0.5)
Intine thickness	1.1(0.8-1.4)	1.3 (1.1-1.5)	1.15(1.0-1.3)	1.05 (0.9-1.2)	1.3 (1.1-1.5)
Pollen fertility	78.66%	94.85%	61.42%	90.22%	79.83%
Shape in polar view	Semi-angular, prolate, spheroidal	Circular and semi-circular	Circular, semicircular and tubular	Circular	Circular
Shape in equatorial view	circular and semi-circular	Semi-angular and angular	Circular	Sub-prolate, prolate and spheroidal	Circular, semi-circular and sub-prolate
Position of pore	Endoporus	Endoporus	Endoporus	Endoporus	Ectoporus

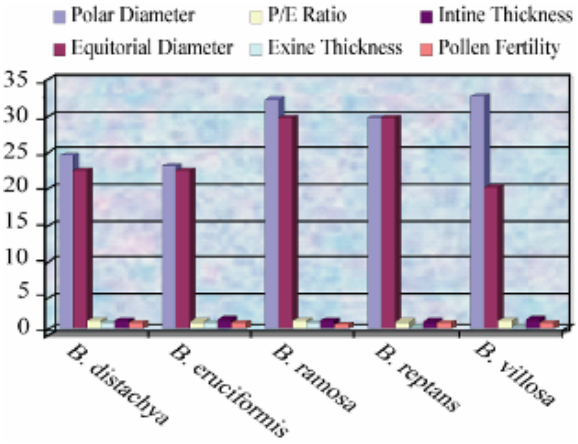

Fig. 6. Summary of pollen measurements of *Brachiaria*.

Conclusion

It is proved during this study that most of the palynological characters are stable in most of the species of genus *Brachiaria*. It is clear from the dendrogram that palynological studies are very significant to classify and delimit taxa. During this study it is also proved that although *Brachiaria villosa* was given in the Flora of Pakistan (1990) but was considered to be of doubtful origin in Pakistan by Stewart. This species is also confirmed to occur in Pakistan in the present study. Also there is no published data already regarding the morpho-palynological distinctions among the species of *Brachiaria* particularly from Pakistan.

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(Received for publication 1 September 2012)