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Numerical Taxonomic Study of Some Tribes of Gramineae from Egypt

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ABSTRACT

A systematic study of eleven tribes of Gramineae surveyed 34 *characters including fruit morphology*, *fruit anatomy and palynology. The results were conducted to some numerical analysis aspects. On the basis of UPGMA* (*Unpaired Group Method of Average*) *clustering and PCA* (*Principal component analysis*), *the results show congruence between the UPGMA clustering and PCA method*, *in suggesting two major clads/groups and five subclads.*

***Keywords*: ** *Gramineae*, *Numerical Taxonomy*, *UPGMA*, *Cladistic Tree*

1. Introduction

Poaceae (grasses) is one of the most species-rich flowering plant families and includes many economically important crops. Parallel evolution of such features as the annual habit, C4 photosynthesis and several highly characteristic reproductive structures has facilitated a series of major radiations within Poaceae, culminating in the existing global distribution of about 10000 species and 700 genera [1,2]. A phylogeny of Poaceae was recently established using a combination of multiple data sets from both molecules and morphology [3], enabling improved understanding of relationships between basal and derived grasses.

Poaceae tribes and genera are subject to different studies in order to understand the phylogenetic relationships between taxa. Many attempts have been made to address phylogenetic relationships of Chloridoideae; synonym Eragrostoideae that comprises approximately 146 genera and 1360 species, whose adoption of efficient C4 photosynthesis had led to its successful proliferation in the tropics and subtropics [1]; mainly based on the basis of morphological and molecular data [4,5], but general agreement is still lacking. The grass tribe Triticeae includes some of the world's most important cereals and a significant number of important forage grasses [6]. Due to its renownedly complicated evolutionary history and its economic importance there has been an increasing interest in producing molecular phylogenies for the Triticeae. Attempts to unravel the relationships in the group have been based on many different types of data e.g. isozymes [7], restriction site data [8,9] and sequence data from a number of different coding and/or non-coding regions, viz.5S RNA [10]. Among the modern tools for plant taxonomy, reference [11] stated that increasing use has taken place of computers for data storage and analysis during the past twenty years. Data derived from all tools of taxonomical investigations has to be analyzed mathematically and cladistic trees have to be drawn. Despite of the criticism of using cladistic analysis in taxonomy, cladistic methods have become a most useful technical tool for clarifying intrafamilial relationships. Moreover; the advantages of using more rigorous techniques to elaborate natural classifications or evolutionary diagrams instead of those that have been used traditionally in botany have been well presented by [12]. A phylogenetic analysis of Triticeae was performed by means of numerical methods due to [13]. Five methods, each based on extreme assumptions of parameters so interpreted under [14] evolutionary model, were used. The most parsimonious tree obtained served as a base for subsequent elaboration of the final tree, taking into consideration genetic information primarily, and for the erection of the proposed phylogenetic classification of Triticeae. A key is provided for identification of the groupings in the tribe. The proposed classification is discussed in the light of previous classifications, even though none of them were phylogenetic in the sense of Hennig. Reference [15] have introduced a cladistic analysis, primarily based on morphological data from 40 taxa representing the 24 genera of the Triticeae. They used Bremer support as a measure of branch support. The trees based on morphology and on molecular data are largely incongruent. Also; [16] and [17]; in their study showed the relationships of graminid/restiid of poales in a cladistic tree. This report aims to apply numerical taxonomy; UPGMA and Cluster Analysis; to reveal better the relationships between genera within a tribe and tribes within the family based on the data collected from the previous investigations for caryopses morphology and anatomy and pollen grains morphology.

2. Materials and Methods

2.1. Plant Material

The study dealt with 34 species belonging to 25 genera of 11 tribes of Gramineae; Andropogoneae, Aristideae, Arundineae, Aveneae, Brachypodieae, Bromeae, Eragrostideae, Paniceae, Poeae, Stipeae and Triticeae. The study based on herbarial specimens dried and kept in the QNA Herbarium (in South Valley University, Qena, Botany Department) and some species received on loan from CAI Herbarium for the palynological study (**Table 1**). In the following analysis, species and genera constituted the OTUs (Operational Taxonomic Units). In order to broadly sample variation, the OUTs consist of a number of collections from different localities in Egypt, illustrated in **Table 2**.

2.2. Characters Observations

Table 3 shows the characters and character states scored for fruit anatomy, fruit morphology and pollen morphology, averaged for each OUT. A total of 37 characters were measured, comprising 22 qualitative and 15 quantitative characters. For recording the total characters; a main using of different microscopic techniques; light, scanning electron and stereomicroscope were used for investigating different samples and recording data collected. **Table 4** shows the data matrix used for analysis of taxa studied. For some of the OTUs, some characters' observations were lacked and these omissions were coded as missing data (−0.999).

2.3. Data Analysis

Two types of analysis were performed with STATISTICA version 5.0 computer software. Firstly, the total data coded were analyzed by the Unpired Group Method of Average (UPGMA) clustering. Construction of the tree illustrating the relationships between the studied species was performed using Arthimetic Average (UPGMA) proposed by [18]. Secondly, factor analysis and factor loadings were applied to determine the major and specific characters that aid in separation using the same program. A principal component analysis (PCA) was also performed according to [19].

3. Results

Figure 1 shows the UPGMA cladistic tree comprising all OTUs in the present study. The tree separated into two major clads at 100 dissimilarity distance. The first major clad at 53 dissimilarity distance, comprised only two species of the total number; Panicum turgidum and Arundo donax; while the second major clad at 93 dissimilarity comprised the rest 32 species.

The second major clad separated into two branches, the first branch includes five subclads: 1) A subclad at 86 dissimilarity distance with five species; Lamarckia aurea, Oryzopsis miliacae, Polypogon monspeliensis, Eragrostis cilinensis and Stipagrostis ciliata. 2) A subclad at 84 dissimilarity distance with Aegilops kotshyi. 3) A subclad at 80 dissimilarity distance comprises six species; Aegilops ventricosa, Hordeum murinum ssp. leporinum, Lolium perenne, Bromus scoparius, Brachypodium distachyum and Avena fatua. 4) A subclad at 74 dissimilarity distance includes nine species; Stipa capensis, Dactylis glomerata, Stipa lagascae, Bromus rubens, Echinocoloa colona, Coelachryum bervifolium, Schismus arabicus, Stipa parviflora and Aristida funiculata and 5) A subclad at 54 dissimilarity distance with nine species, Poa annua, Polypogon maritimus, Eragrostis minor, Phalaris minor, Avena barbata, Aristida mutabilis, Cenchrus ciliaris, Leptochloa fusca and Aristida adscensionis. The second branch of the second major clad comprises two species; Dactylochtenium aegyptium and Sorghum variegatum; at 66 dissimilarity distance.

Factor analysis using Principal Component Analysis (PCA) showed that the most intrinsic characters enhanced separation of the total OTUs are fruit shape, color type and fruit surface sculpture of the morphological characters, of the fruit anatomical characters; section outline shape, hull cells type, aleurone cells shape and orientation, scutellum shape and thickness and endosperm thickness are intrinsic characters for separation. Meanwhile, all the pollen characters are good data for separating of taxa; pollen class, shape, size, surface sculpture, annulus thickness, pore diameter, pollen wall thickness, sexine and nexine thickness. The characters of separation are of high factor loadings ≥ (± 0.7) Table 5. These represented by a percentage of the total variation as 24.01% from the three factors extracted as; factor 1 is responsible for 16.54% of the variation, factor 2 is responsible for 4.34% of the variation and factor 3 is responsible for the minimum value of the total variation; is 3.13%. The plot of 34 OTUs on the first two factors extracted in the PCA method is shown in (**Figure 2**). Plot of factor 1/2 shows two groups. 1) Group of 6. Arundo

Table 1. List for the investigated taxa with their geographical region.

No.	Species	Herb.	Collecting region	Year	Collector
1	*Sorghum varigatum*	QNA	N	2004	A.K. OSMAN
2	*Aristida adscensionis*	QNA	GE	2004	A.K. OSMAN
3	*Aristida funiculate*	QNA	GE	2004	A.K. OSMAN
4	*Aristida mutabilis*	QNA	GE	2004	A.K. OSMAN
5	*Stipagrostis ciliata*	QNA	R	2004	A.K. OSMAN
6	*Arundo donax*	QNA	N	2009	N.R.A. HUSSEIN
7	*Schismus arabicus*	QNA	M	2006	A.K. OSMAN
8	*Avena barbata*	QNA	N	2005	A.K. OSMAN
9	*Avena fatua*	QNA	N	2005	A.K. OSMAN
10	*Phalaris minor*	QNA	M	2006	A.K. OSMAN
11	*Polypogon maritimus*	QNA	N	2006	A.K. OSMAN
12	*Polypogon monspeliensis*	QNA	N	2006,07	A.K. OSMAN
13	*Brachypodium distachym*	QNA	M	2006	A.K. OSMAN
14	*Bromus rubens*	QNA	M	2005	A.K. OSMAN
15	*Bromus scoparius*	QNA	M	2006	A.K. OSMAN
16	*Coelachyrum bervifolim*	QNA	GE	2004	A.K. OSMAN
17	*Dactylochtenium aegyptium*	QNA	N	2006	A.K. OSMAN
18	*Eragrostis cilianensis*	QNA	S	2005	A.K. OSMAN
19	*Eragrostis minor*	QNA	M	2005	A.K. OSMAN
20	*Leptochloa fusca*	QNA	S	2005	A.K. OSMAN
21	*Cenchrus ciliaris*	QNA	GE	2004	A.K. OSMAN
22	*Echinochloa colona*	QNA	N	2006	A.K. OSMAN
23	*Panicum turgidum*	QNA	N	2005	A.K. OSMAN
24	*Dactylis glomerata*	QNA	M	2006	A.K. OSMAN
25	*Lamarckia aurea*	QNA	M	2006	A.K. OSMAN
26	*Lolium perenne*	QNA	N	2009	N.R.A. HUSSEIN
27	*Poa annua*	QNA	N	2005	A.K. OSMAN
28	*Oryzopsis miliacea*	CAI	M	2006	A.K. OSMAN
29	*Stipa capensis*	QNA	M	2006	A.K. OSMAN
30	*Stipa lagascae*	QNA	M	2006	A.K. OSMAN
31	*Stipa parviflora*	QNA	M	2006	A.K. OSMAN
32	*Aegilops kotshyi*	QNA	M	2006	A.K. OSMAN
33	*Aegilops ventricosa*	CAI	M	2006	A.K. OSMAN
34	*Hordium murinum Subsp. Leporinum*	CAI	S	2005	A.K. OSMAN

QNA = Qena Faculty of Science Herbarium (QNA a proposed Agronym); M = Mediterranean region; N = Nile region; R = Red sea coastal region; S = Sinai; GE= Gabel Elba.

Table 2. List for the names of total OTUs studied and their corresponding numbers and tribe names.

OTUs no.	Species name	Tribes	OTUs no.	Species name	Tribes
Sp.1	*Sorghum varigatum*	Andro	**Sp.18**	*Eragrostis cilianensis*	Eragro
Sp.2	*Aristida adscensionis*	Arist	**Sp.19**	*Eragrostis minor*	Eragro
Sp.3	*Aristida funiculate*	Arist	**Sp.20**	*Leptochloa fusca*	Eragro
Sp.4	*Aristida mutabilis*	Arist	**Sp.21**	*Cenchrus ciliaris*	Panic
Sp.5	*Stipagrostis ciliate*	Arist	**Sp.22**	*Echinochloa colona*	Panic
Sp.6	*Arundo donax*	Arund	**Sp.23**	*Panicum turgidum*	Panic
Sp.7	*Schismus arabicus*	Arund	**Sp.24**	*Dactylis glomerata*	Poeae
Sp.8	*Avena barbata*	Aven	**Sp.25**	*Lamarckia aurea*	Poeae
Sp.9	*Avena fatua*	Aven	**Sp.26**	*Lolium perenne*	Poeae
Sp.10	*Phalaris minor*	Aven	**Sp.27**	*Poa annua*	Poeae
Sp.11	*Polypogon maritimus*	Aven	**Sp.28**	*Oryzopsis miliacea*	Stipeae
Sp.12	*Polypogon monspeliensis*	Aven	**Sp.29**	*Stipa capensis*	Stipeae
Sp.13	*Brachypodium distachym*	Brach	**Sp.30**	*Stipa lagascae*	Stipeae
Sp.14	*Bromus rubens*	Brom	**Sp.31**	*Stipa parviflora*	Stipeae
Sp.15	*Bromus scoparius*	Brom	**Sp.32**	*Aegilops kotshyi*	Triti
Sp.16	*Coelachyrum bervifolim*	Eragro	**Sp.33**	*Aegilops ventricosa*	Triti
Sp.17	*Dactylochtenium aegyptium*	Eragro	**Sp.34**	*Hordium murinum Subsp. Leporinum*	Triti

Andro= Andropogoneae, Arist= Aristideae, Arund= Arundineae, Aven= Aveneae, Brach = Brachypodieae, Brom= Bromeae, Eragro= Eragrostideae, Panic= Paniceae,Triti= Triticeae.

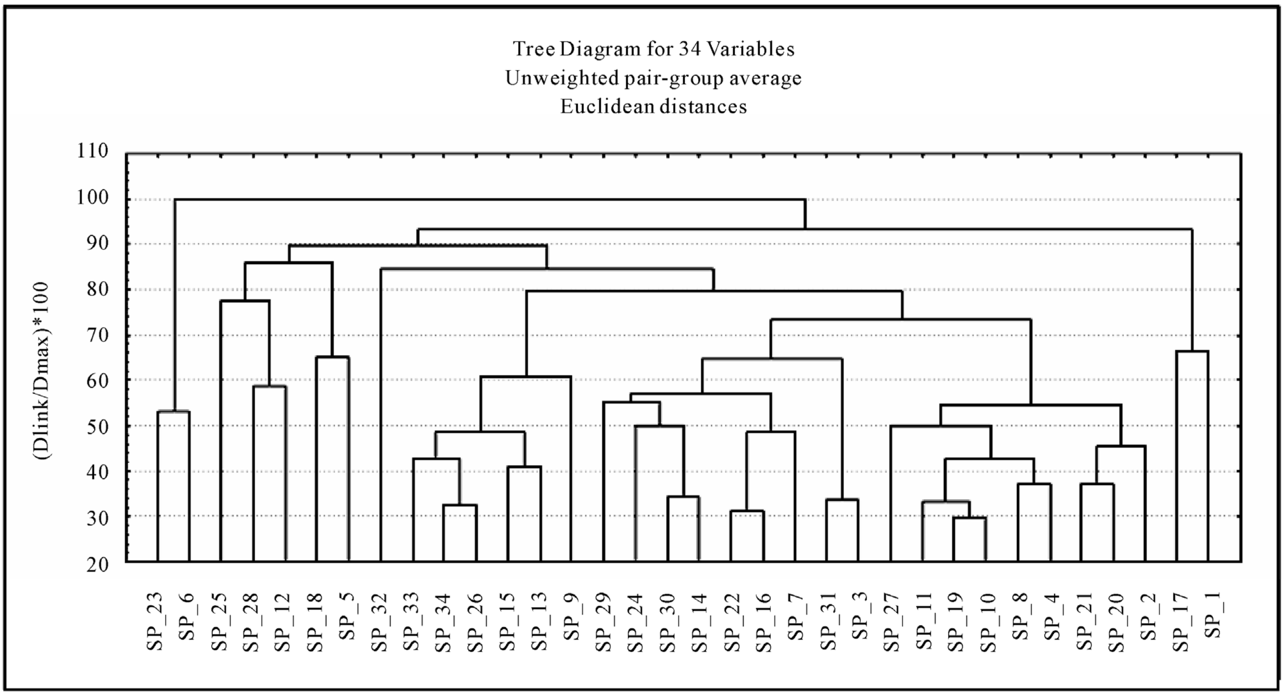

Figure 1. Cladogram of 34 species studied by UPGMA method.

Table 3. Characters and character states used in the analysis of Gramineae tribes.

Characters	Character states	Code
	Fruits Morphological characters	
1. Fruit shape	Elliptic	1
	Rectangular	2
	Cordate	3
	Oblong	4
	Linear	5
	Narrow cordate	6
	Cordate with hollow part	7
	Circular	8
	Oval	9
	Oval with acute protrusion	10
	(tall) oblong with tapered ends	11
	Elliptic with tapered ends (acute elliptic)	12
2. Fruit coloring	Onecoloured (uniformly coloured)	1
	Bicoloured	2
3. Color type	Light brown	1
	Dark brown with light yellowish sheath	2
	Brown	3
	Beage	4
	Dark red	5
	Light beage	6
	Light beage and light green strips	7
	Brown with pale beage sheath	8
	Beage with light violet sheath	9
	Brown and light orange	10
	Beage and brown protrusions	11
	Shiny beage and light brown small spots	12
	Dark beage	13
	Beage and brown black spots	14
	Light brown and dark brown ends	15
	Light brown with light green sheath	16
	Brown with light green sheath	17
	Gradient beage with light green and brown spot at grain top	18
	Trichomes on grains	
4. Trichomes type	Simple hairs	1
5. Trichome presence	Absent (glabrous)	1
	Present	2
	Present on grain sheath	3
6. Hair length	Short	1
	Long	2
	With different lengths	3
7. Hair coloring	Shiny transparent (colorless)	1
	Colored	2
8. Position of attaching	Around all grain surface	1
	At grain edges	2
	Around all surface, condensed at the top	3
	At the top	4
	At the base	5
	Few around all surface, condensed at the base	6
	On the side margins of the grain sheath	7
9. Fruit surface sculpture	Reticulate with straight cell wall	1
	Striate	2
	Scabrate	3

	Reticulate with undulate granulate cell wall	4
	Compound reticulate with foveolate	5
	Reticulate with undulate cell wall	6
	Striate to scabrate	7
	Scalariform	8
	Striate at the intermediate and reticulate with straight cell wall next to hilum	9
	Compound reticulate with tubrculate	10
	Smooth	11
	Rugose	12
	Reticulate with straight to undulate cell wall	13
	Ribbed pattern cell wall	14
	Compound reticulate with granulate	15
	Scaly surface	16
	Fruits Morph metrical characters	
10. Fruit weight (mg)	=(0.03-2.12)	1
	=(2.12-4.21)	2
	=(4.21-6.3)	3
	=(6.3-8.39)	4
	=(8.39-10.48)	5
	=(10.48-12.57)	6
	=(12.57-14.66)	7
	Fruit size	
11. Wide (µm)	=(29-112)	1
	=(112-195)	2
	=(195-279)	3
12. Length (µm)	=(1.9-52.9)	1
	=(52.9-103.9)	2
	=(103.9-154.9)	3
	=(154.9-205.9)	4
	=(205.9-256.9)	5
	=(256.9-307.94)	6
	=(307.94-358.9)	7
	Fruit anatomy	
13. Section outline shape	Circular	1
	Circular to cordate	2
	Circular to oval	3
	Semi-circular	4
	Oval	5
	Oval to cordate	6
	Cordate	7
	Oval to rectangular with curved corners	8
	Oval to rectangular	9
	C- shaped	10
	Circular to triangular with obtused corners	11
	Triangular with obtused corners	12
	Triangular	13
	Rectangular with obtused corners	14
	Rectangular with obtused corners to oval	15
	Rectangular with acuted corners	16
	Rectangular	17
	Polygonal	18
	Linear with folded ends	19
	Circled linear	20
14. Hull cells type	Parenchyma	1
	Epithelial	2
	Not observed	3

15. Aleurone cells shape	Rectangular	1
	Rectangular and quadrate	2
	Quadrate	3
	Rectangular and polygonal	4
	Not observed	5
16. Aleurone cells oreintation	Horizontal	1
	Vertical	2
	Horizontal and vertical	3
	Not observed	4
17. Scutellum cells shape	Strip of cells	1
	Elliptic mass of cells	2
	Rectangular to cordate mass of cells	3
	Quinqangular mass of cells	4
	Oval mass and strip of cells	5
	Triangular mass and strip of cells	6
	Not observed	7
18. Endosperm differentiation	Differentiated	1
	Differentiated and Undifferentiated	2
19. Type of endosperm	Starchy	1
	Starchy and fluidy	2
20. Section wide (µm)	=(5.33-31.66)	1
	=(31.66-57.99)	2
	=(57.99-84.32)	3
	=(84.32-110.65)	4
	=110.65-136.98)	5
	=(136.98-163.31)	6
	=(163.31-189.64)	7
21. Section length (µm)	=(33.33-71.94)	1
	=(71.94-110.55)	2
	=(110.55-149.16)	3
	=(149.16-187.77)	4
	=(187.77-226.38)	5
	=(226.38-264.99)	6
	=(264.99-303.6)	7
22. Hull cells thickness (µm)	Not ovserved	0
	=(9.39-14.79)	1
	=(14.79-20.19)	2
	=(20.19-25.59)	3
	=(25.59-30.99)	4
	=(30.99-36.39)	5
23. Seed coat thickness (µm)	=(2.43-8.63)	1
	=(8.63-14.83)	2
	=(14.83-21.03)	3
	=(21.03-27.23)	4
	=(27.23-33.43)	5
	=(33.43-39.63)	6
24. Aleurone layer thickness (µm)	Not observed	0
	=(1.92-9.42)	1
	=(9.42-16.92)	2
	=(19.92-24.42)	3
	=(24.42-31.92)	4
	=(31.92-39.42)	5
	=(39.42-46.92)	6
25. Scutellum thickness (µm)	Not observed	0
	=(5.54-85.94)	1
	=(85.94-166.43)	2
	=(166.34-246.74)	3

	=(246.74-327.14)	4
	=(327.14-407.54)	5
	=(407.54-487.94)	6
	Thicknesses in the range codes 1&4	7
	Thicknesses in the range codes 1&3	8
26. Endosperm thickness (μm)	=(21.52-125.02)	1
	=(125.02-228.52)	2
	=(228.52-332.02)	3
	=(332.02-435.52)	4
	=(435.52-539.02)	5
	=(539.02-642.52)	6
	Two different thicknesses in range of codes 1&2	7
	Pollen grains Morphological characters	
27. Pollen class	Monoporate	1
	Diporate	2
28. Annulus	Pollen annulate	1
29. Operculum	Pollen operculate	1
30. Pollen size	Pollen small	1
	Pollen medium	2
31. Pollen shape	Pollen Oblate-spheroidal	1
	Pollen Suboblate	2
	Pollen Prolate-spheroidal	3
	Pollen spheroidal	4
32. Surface sculpturing	Areolate	1
	Granulate	2
	Scabrate	3
	verrucate	4
	Pollen grains Morph metrical characters	
33. Annulus thickness (μm)	=(1.16-1.52)	1
	=(1.52-1.88)	2
	=(1.88-2.24)	3
	=(2.24-2.6)	4
	=(2.6-2.96)	5
	=(2.96-3.32)	6
34. Pore diameter (μm)	=(1.57-2.17)	1
	=(2.17-2.77)	2
	=(2.77-3.37)	3
	=(3.37-3.97)	4
	=(3.97-4.57)	5
35. Pollen wall thickness (μm)	=(0.698-0.848)	1
	=(0.848-0.998)	2
	=(0.998-1.148)	3
	=(1.148-1.298)	4
	=(1.298-1.448)	5
36. Sexine thickness (μm)	=(0.434-0.454)	1
	=(0.454-0.474)	2
	=(0.474-0.494)	3
	=(0.494-0.514)	4
	=(0.514-0.534)	5
	=(0.534-0.554)	6
37. Nexine thickness (μm)	=(0.314-0.354)	1
	=(0.354-0.394)	2
	=(0.394-0.434)	3
	=(0.434-0.474)	4
	=(0.474-0.514)	5
	=(0.514-0.554)	6

Table 4. Data matrix used in the numerical analysis for some Gramineae tribes.

	Sp.1	Sp.2	Sp.3	Sp.4	Sp.5	Sp.6	Sp.7	Sp.8	Sp.9	Sp.10	Sp.11	Sp.12	Sp.13	Sp.14	Sp.15	Sp.16
1	3	5	5	5	5	−0.999	9	4	4	3	3	4	5	11	5	7
2	2	1	1	1	1	−0.999	2	1	1	1	1	1	1	1	1	1
3	18	9	8	3	1	−0.999	12	6	4	3	1	16	5	5	5	5
4	0	1	1	1	1	−0.999	0	1	1	1	0	0	1	1	1	0
5	0	1	1	1	1	−0.999	0	1	2	1	0	0	1	1	1	0
6	0	2	2	2	2	−0.999	0	2	2	1	0	0	1	1	1	0
7	0	5	5	5	5	−0.999	0	3	1	1	0	0	2	1	7	0
8	2	7	6	2	14	−0.999	11	2	2	3	7	12	2	15	2	6
9	1	1	1	1	1	−0.999	1	1	4	1	1	1	2	2	1	1
10	3	3	1	1	1	−0.999	1	2	5	3	1	1	2	3	1	2
11	1	3	1	1	1	−0.999	1	1	4	1	1	1	1	1	2	1
12	16	5	20	4	1	−0.999	12	6	17	5	1	2	10	15	10	10
13	1	1	3	2	2	−0.999	3	3	3	3	3	1	3	3	3	1
14	1	1	5	1	1	−0.999	1	1	1	4	2	1	1	2	1	1
15	1	1	4	3	1	−0.999	1	2	2	2	2	2	1	2	2	1
16	2	2	7	2	1	−0.999	1	4	1	1	1	7	7	7	7	1
17	1	1	1	1	1	−0.999	1	1	1	1	1	1	2	1	1	1
18	1	1	2	1	1	−0.999	1	1	1	1	1	1	1	1	1	1
19	2	1	2	2	2	−0.999	2	4	4	2	2	1	2	2	1	2
20	3	1	1	1	1	−0.999	1	3	5	2	1	1	3	2	2	2
21	1	1	0	1	1	−0.999	0	0	0	0	0	2	0	0	0	3
22	2	1	1	1	1	−0.999	2	1	4	2	1	1	2	1	2	2
23	1	1	0	1	1	−0.999	1	5	5	3	2	2	1	3	3	1
24	2	2	0	2	1	−0.999	1	5	1	1	1	0	0	0	0	1
25	1	1	1	2	4	−0.999	3	5	6	4	4	2	7	2	1	1
26	1	−0.999	−0.999	−0.999	1	1	−0.999	−0.999	1	−0.999	−0.999	−0.999	2	−0.999	2	−0.999
27	2	−0.999	−0.999	−0.999	1	2	−0.999	−0.999	2	−0.999	−0.999	−0.999	2	−0.999	2	−0.999
28	2	−0.999	−0.999	−0.999	4	1	−0.999	−0.999	1	−0.999	−0.999	−0.999	4	−0.999	4	−0.999
29	3	−0.999	−0.999	−0.999	2	1	−0.999	−0.999	3	−0.999	−0.999	−0.999	2	−0.999	2	−0.999
30	2	−0.999	−0.999	−0.999	3	2	−0.999	−0.999	3	−0.999	−0.999	−0.999	1	−0.999	4	−0.999
31	1	−0.999	−0.999	−0.999	1	2	−0.999	−0.999	4	−0.999	−0.999	−0.999	2	−0.999	2	−0.999
32	5	−0.999	−0.999	−0.999	1	1	−0.999	−0.999	1	−0.999	−0.999	−0.999	1	−0.999	2	−0.999
33	2	−0.999	−0.999	−0.999	1	2	−0.999	−0.999	1	−0.999	−0.999	−0.999	2	−0.999	2	−0.999
34	3	−0.999	−0.999	−0.999	4	3	−0.999	−0.999	3	−0.999	−0.999	−0.999	2	−0.999	3	−0.999

	Sp.17	Sp. 18	Sp. 19	Sp. 20	Sp. 21	Sp. 22	Sp. 23	Sp. 24	Sp. 25	Sp. 26	Sp. 27	Sp. 28	Sp. 29	Sp. 30	Sp. 31	Sp. 32	Sp. 33	Sp. 34
1	9	8	1	1	3	3	6	10	12	2	1	9	4	5	5	4	2	4
2	2	1	1	2	2	2	1	1	2	1	1	1	1	1	1	1	1	1
3	11	3	1	10	14	7	4	3	15	1	3	13	1	3	4	17	1	4
4	0	0	0	0	0	0	0	0	1	0	1	1	2	1	1	1	1	0
5	0	0	0	0	0	0	0	0	2	0	1	1	3	1	1	1	1	0
6	0	0	0	0	0	0	0	0	2	0	2	2	2	2	1	2	2	0
7	0	0	0	0	0	0	0	0	5	0	4	4	6	1	5	4	4	0
8	10	16	1	1	6	5	1	6	6	2	8	12	9	13	2	2	2	4
9	1	1	1	1	1	1	1	1	1	2	1	1	1	1	2	3	5	2
10	1	1	1	1	2	3	5	1	2	3	1	2	1	1	2	4	5	2
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1
12	18	1	2	1	5	13	−0.999	11	−0.999	8	3	1	14	14	19	7	9	8
13	3	3	3	3	3	1	−0.999	3	−0.999	3	3	3	3	3	3	3	3	3
14	1	1	5	1	1	1	−0.999	5	−0.999	1	3	3	1	2	5	1	2	1
15	1	1	4	1	1	1	−0.999	4	−0.999	2	1	1	1	1	4	1	2	2
16	6	5	1	1	1	1	−0.999	7	-0.999	7	7	7	1	7	7	3	7	7
17	1	1	1	1	1	1	−0.999	1	−0.999	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	−0.999	1	−0.999	1	1	1	1	1	1	1	1	1
19	2	2	2	1	2	2	−0.999	3	−0.999	3	2	2	1	2	1	6	5	6
20	1	1	1	1	3	1	−0.999	1	−0.999	3	1	1	1	1	2	5	6	4
21	0	0	0	0	0	5	−0.999	0	−0.999	0	0	0	0	0	0	0	0	0
22	2	1	2	1	1	1	−0.999	1	−0.999	4	6	6	1	2	2	3	6	5
23	1	1	0	1	1	2	−0.999	0	−0.999	5	2	2	1	1	0	5	4	6
24	7	8	1	1	1	1	−0.999	0	−0.999	0	0	0	1	0	0	6	0	0
25	2	3	4	3	4	4	−0.999	4	−0.999	3	3	3	5	3	1	5	6	3
26	2	−0.999	−0.999	−0.999	−0.999	−0.999	1	−0.999	−0.999	1	−0.999	1	−0.999	−0.999	−0.999	−0.999	2	2
27	2	−0.999	−0.999	−0.999	−0.999	−0.999	2	−0.999	−0.999	2	−0.999	2	−0.999	−0.999	−0.999	−0.999	2	2
28	3	−0.999	−0.999	−0.999	−0.999	−0.999	1	−0.999	−0.999	4	−0.999	1	−0.999	−0.999	−0.999	−0.999	4	1
29	2	−0.999	−0.999	−0.999	−0.999	−0.999	1	−0.999	−0.999	4	−0.999	2	−0.999	−0.999	−0.999	−0.999	1	2
30	2	−0.999	−0.999	−0.999	−0.999	−0.999	4	−0.999	−0.999	3	−0.999	1	−0.999	−0.999	−0.999	−0.999	5	3
31	1	−0.999	−0.999	−0.999	−0.999	−0.999	1	−0.999	−0.999	4	−0.999	2	−0.999	−0.999	−0.999	−0.999	3	2
32	3	−0.999	−0.999	−0.999	−0.999	−0.999	2	−0.999	−0.999	3	−0.999	2	−0.999	−0.999	−0.999	−0.999	4	1
33	2	−0.999	−0.999	−0.999	−0.999	−0.999	2	−0.999	−0.999	2	−0.999	2	−0.999	−0.999	−0.999	−0.999	2	2
34	3	−0.999	−0.999	−0.999	−0.999	−0.999	1	−0.999	−0.999	3	−0.999	3	−0.999	−0.999	−0.999	−0.999	3	2

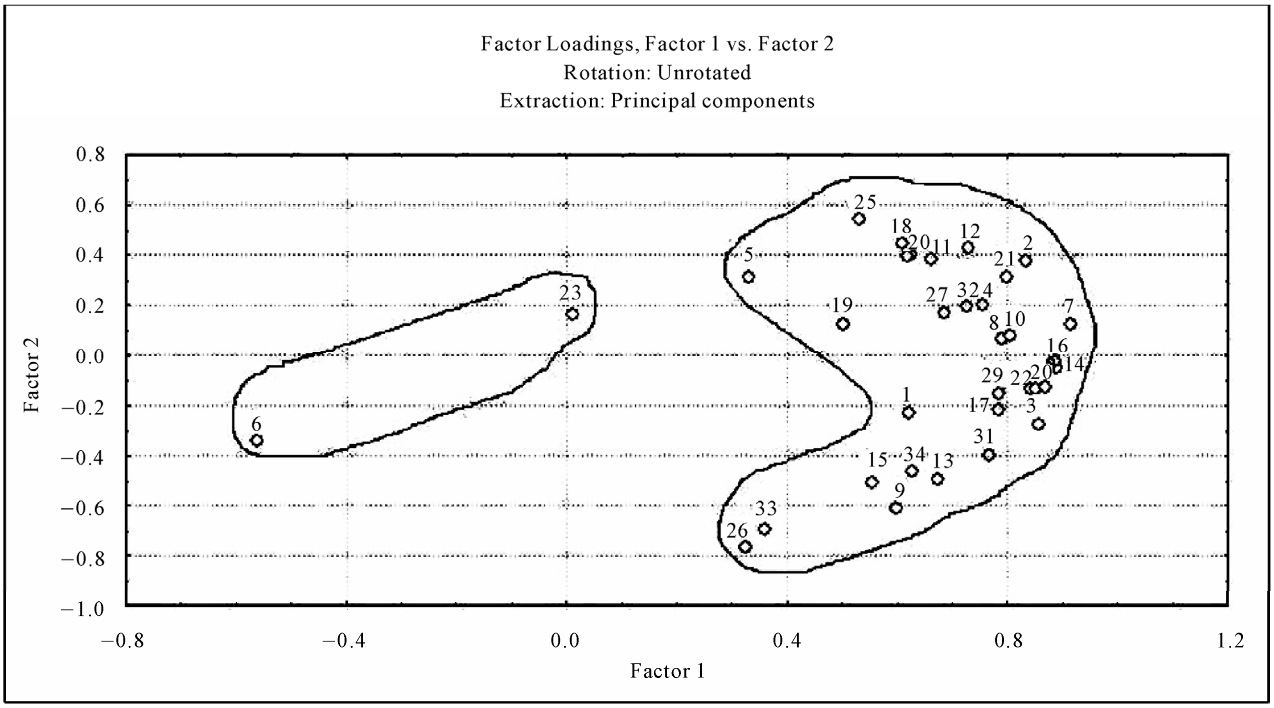

Figure 2. Scatter-plot of 34 studied taxa plotted against the first factor by the second factor.

donax and 23. Panicum turgidum. 2) Group including the rest 32 species. There are some characters; character of trichomes type, annulus of the pollen and also the operculum of the pollen grain (**Table 3**); which not been fitted into the data matrix because they are of only one code, so they were excluded in the analysis because they had no variation in the matrix.

4. Discussion

In the present study a large number of grains macro- and micro-morphological, anatomical and pollen grains characters were scored and numerical methods (UPGMA and PCA) were applied to study the relationship among eleven Poaceae tribes and estimate the level of variation within and among these tribes. UPGMA gives insight into degree of similarity among the studied species and whether they form groups // clusters and gives an indication of the level of variation within and between tribes. PCA reflects which characters are important on the axes, and indicates the significant characters based on the highest factor score (**Table 5**). Therefore it becomes clear which characters cause the separation between groups and can be useful to distinguish taxa. Pollen grains showed the most powerful significant characters, whereas all characters have been recorded are of high factor scores. Generally, our results show congruence between the UPGMA clustering and PCA analysis in suggesting two main groups and five subgroups which included the distribution of eleven tribes studied.

Our UPGMA results show that the tribe Andropogoneae is separated in one branch of the cladistic tree, the tribe Aristidieae is separated in three branches of the tree through three different subclads // subgroups. The tribe Arundineae with two species is separated in two branches of different clads in the tree. The tribe Aveneae is separated in five branches of the tree while the tribe Brachypodieae is separated in one branch and the Bromeae is separated in two branches within two different subclads. The Eragrostideae separated in four branches and the Paniceae separated in three branches. The Stipeae separated in four branches in only two clads while the Triticeae separated in three branches in also two clads. All the mentioned species, tribes, major clades and subclads are arranged as the following:

The first main clads // groups of two species 6. Arundo donax (Tribe: Arundineae) and 23. Panicum turgidum (Tribe: Paniceae). While the second main group includes a large variety of taxa from different tribes; 32 species of tribes: Andropogoneae, Aristideae, Arundineae, Aveneae, Brachypodieae, Bromeae, Eragrostideae, Paniceae, Stipeae and Triticeae. These tribes are separated through five distinct subgroups: 1) Sub-clad of species 25. Lamarckia aurea, 28. Oryzopsis miliacea, 12. Polypogon monspeliensis, 18. Eragrostis cilianensis and 5. Stipagrostis ciliata belonging to Poeae, Stipeae, Aveneae, Eragrostideae, and Aristideae. 2) Sub-clad only of species

Table 5. Factor loadings showed the most intrinsic characters enhanced separations of the studied species.

	Characters	Factor Loadings Rotation: Un-rotated Extraction: Principal components		
		Factor 1	Factor 2	Factor 3
1.	**Fruit shape**	**1.578348**	**0.871665**	**−1.24653**
2.	**Coloring mode**	−0.23281	0.691848	0.173174
3.	**Color type**	**2.27393**	**2.051546**	**−3.32845**
4.	**Trichome presence**	−0.54117	0.571291	0.467989
5.	**Hair length**	−0.50498	0.545862	0.444604
6.	**Hair coloring**	−0.36187	0.617713	0.489408
7.	**Position of attaching**	0.194314	0.461728	0.273855
8.	**Fruit surface sculpture**	**2.058189**	**2.095991**	−0.0891
9.	**Fruit weight (mg)**	−0.15868	−0.01955	0.347091
10.	**Fruit wide (mm)**	0.125955	0.069094	−0.25377
11.	**Fruit length (mm)**	−0.18849	0.39183	0.305645
12.	**Section outline shape**	**3.173544**	**−3.89842**	−0.03563
13.	**Hull cells type**	0.443799	0.150657	**1.117598**
14.	**Aleurone cells shape**	0.12138	0.430593	**1.567286**
15.	**Aleurone cells orientation**	0.052685	0.232461	**1.360735**
16.	**Scutellum shape**	**1.010615**	**−1.21997**	0.271807
17.	**Endosperm differentiation**	−0.33176	0.452823	0.673013
18.	**Endosperm type**	−0.33784	0.490211	0.698834
19.	**Section wide (µm)**	0.296755	−0.18378	0.934019
20.	**Section length (µm)**	0.097103	−0.48171	0.569057
21.	**Hull cells thickness (µm)**	−0.60307	0.690619	0.471997
22.	**Seed coat thickness (µm)**	0.18211	−0.36414	0.73636
23.	**Aleurone layer thickness (µm)**	0.130375	−0.39784	0.654374
24.	**Scutellum cells thickness (µm)**	−0.18054	**1.09338**	0.69615
25.	**Endosperm thickness (µm)**	**0.764397**	−0.05966	**1.460169**
26.	**Pollen class**	**−1.07075**	−0.2744	−0.64054
27.	**Pollen size**	**−1.05331**	−0.44013	**−1.01514**
28.	**Pollen shape**	**−0.92265**	**−0.79847**	**−0.89728**
29.	**Pollen surface sculpture**	**−0.98552**	−0.54043	**−0.84148**
30.	**Annulus thickness (µm)**	**−0.96595**	**−0.83463**	**−1.30983**
31.	**Pore diameter (µm)**	**−1.02089**	**−0.78764**	**−0.8329**
32.	**Pollen wall thickness (µm)**	**−1.00316**	−0.51699	**−0.97543**
33.	**Sexine thickness (µm)**	**−1.06557**	−0.39242	**−1.01249**
34.	**Nexine thickness (µm)**	**−0.97452**	−0.69914	**−1.23458**
	Percentage per PCA	**16.54**	**4.34**	**3.13**
	Percentage for total variation for the three factors extracted **24.01 %**			

*PCA: Principal Component Analysis

32. Aegilops kotshyi belongs to Triticeae. 3) Sub-clad of 33. Aegilops ventricosa, 34. Hordium murinum Subsp. Leporinum, 26. Lolium perenne, 15. Bromus scoparius, 13. Brachypodium distachym and 9. Avena fatua belonging to Triticeae, Poeae, Bromeae, Brachypodieae and Aveneae. 4) Sub-clad of species 29. Stipa capensis, 24. Dactylis glomerata, 30. Stipa lagascae, 14. Bromus rubens, 22. Echinochloa colona, 16. Coelachyrum bervifolium, 7. Schismus arabicus, 31. Stipa parviflora and 3. Aristida funiculata belonging to Stipeae, Poeae, Bromeae, Paniceae, Eragrostideae, Arundineae and Aristideae. 5) Sub-clad of species 27. Poa annua, 11. Polypogon maritimus, 19. Eragrostis minor, 10. Phalaris minor, 8. Avena barbata, 4. Aristida mutabilis, 21. Cenchrus ciliaris, 20. Leptochloa fusca and Aristida adscensionis within tribes: Poeae, Aveneae, Eragrostideae, Paniceae and Aristideae.

Several various monophyletic species which regarded as sister-groups are distinct within five subclads mentioned. Firstly, in the tree (**Figure 1**) Paniceae and Arundineae are a two-species sister-group to the rest whole cluster of the tree, on the other hand, Eragrostideae and Andropogoneae are another two-species sister-group within the second branch of the second major clad in the tree. Moreover, other species within Paniceae, Eragrostideae and Arundineae are separated through some different subclads; thus Andropogoneae alone can be conspicuously differentiated from other tribes by means of its characteristic features for the fruit morphology, fruit anatomy and pollen grains morphology. Secondly, different tribes consume sister-grouping within each of the five subclads distinguished. Tribe Poeae conform a monophyletic sister-group in subclad (1) in a cluster of Stipeae, Aveneae, Eragrostideae and Aristideae and in subclad (5) in a cluster of Stipeae, Eragrostideae, Aristideae, Bromeae, Paniceae and Arundineae. Therefore, Poeae is preferably separated from these tribes depending on its own marked pollen grains characters.

Moreover, the Triticeae shows a distinct variation that can aid the comparison of the relationships between Triticeae, Bromeae and Brachypodieae revealed by [16], where they suggested that the Brachypodieae is the sister group of the Triticeae while the Bromeae is the sister group of the Brachypodieae plus the Triticeae. Brachypodium is the sister-group of a clad including both Bromus and the Triticeae. While, [5] illustrated that the relationships between Bromus and the Triticeae is unresolved, so there is a possibility that the Triticeae is a non-monophyletic group. Meanwhile, in our results, the Triticeae is a monophyletic sister-group to the neighboring clad of Triticeae, Poeae, Bromeae, Brachypodieae and Aveneae (sub-clad 3). This clad which can be separated conspicuously through the Aveneae which is a monophyletic branch through Avena fatua, in addition to the separation of Poeae among tribes of subclads 1 and 5. Thus the Triticeae, Bromeae and Brachypodieae are closely related as confirmed by their palynological similarity, in addition to the compatibility of the fruit morphological (**Table 5**) that enhanced the understanding of the degree of similarity between taxa of these tribes. The Stipeae is a sister-group of the sub-clad (4), with excluding tribes Poeae, Bromeae, Paniceae, Arundineae and Eragrostideae from this sub-clad, thus the Stipeae is separated from the Aristideae and also the similarity degree between them can be conducted to the characters of the fruit morphology and pollen grain morphology illustrated in **Table 5**. Therefore, the applied methods of UPGMA and PCA can be used to study the variation within the tribe and the tribes in the family to determine the relationships between genera and tribes. Our results revealed there is a much separation between tribes Andropogoneae, Arundineae, Aristideae, Stipeae, Poeae and Eragrostideae. However, tribes Triticeae, Bromeae and Brachypodieae showed much closer relationships. In addition to the consideration of those tribes Aveneae, Eragrostideae and Stipeae are the most heterogeneous tribes because the taxa of these tribes found to be interspersed with taxa from tribes Poeae, Paniceae and Aristideae.

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