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Comparative anatomy and numerical studies of the genus *Stipagrostis* Nees (Poaceae) in Egypt and their taxonomic significance

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A comparative anatomy of the genus *Stipagrostis* growing in Egypt was conducted using light microscopy and numerical analysis to evaluate their diagnostic value for systematic studies. Descriptions of stem and leaf cross-section: shape, outer frame boundaries, sclerenchyma and vascular bundles, assimilatory tissue, bundle sheath, and bulliform cells are presented. The results of the present study show beneficial data for evaluating the taxonomy of the genus *Stipagrostis* in Egypt at infrageneric, specific, and infraspecific levels. Five shapes were recognized for the stem outline section and three for the leaf blade. Moreover, three types of *Stipagrostis* leaf vascular bundles were identified: primary, secondary, and tertiary, varying in their shapes from elliptical to round. Leaf bulliform cells were located on the adaxial surfaces. They may be regular, irregular, and fan shaped. Our result reveals that the genus *Stipagrostis* is represented by 14 taxa (12 species and two varieties) in Egypt. A diagnostic key for identifying studied taxa based on anatomical investigated leaf and stem characters was presented.

Keywords: *Stipagrostis*; stem; leaf; anatomy; systematic; Egypt

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INTRODUCTION

Poaceae Barnhart (Gramineae Juss) is a large and economically essential flowering plant family. It is the fifth-largest plant family after Asteraceae, Orchidaceae, Fabaceae, and Rubiaceae, with approximately 799 accepted genera and 11506 species (Hsiao et al. 1998, Peterson et al. 2020, POWO, 2024). It covers about 40% of the Earth's surface (Peterson et al. 2010).

The first scientific attempt to divide the family Poaceae was made by Brown (1814), who identified two distinct spikelet types between Pooideae Benth. (Festucoideae Link) and Panicoideae Link. Later, 13 tribes belonging to two subfamilies were recognized by Bentham (1881). Tzvelev (1989) divided the family Poaceae into two subfamilies, while Clayton and Renvoize (1986) classified it into six subfamilies. Recently, Soreng et al. (2022) provided a modern phylogenetic classification of Poaceae based on molecular and morphological studies, recognizing 11,783 species in 12 subfamilies, seven supertribes, 54 tribes, five supersubtribes, 109 subtribes, and 789 accepted genera. Poaceae is cosmopolitan, ranging from the polar circles to the equator, and from the mountains to the sea. It has been estimated that grasses are the main component in some 25% of Earth's vegetation cover (Heywood et al. 2007). In Egypt, Poaceae is the most prominent flowering plant family, with 284 native and naturalized species in 103 genera belonging to 22 tribes and seven subfamilies (Ibrahim et al. 2018, Peterson et al. 2020). The grasses have been influential in Egypt's daily life and economy for over 12,000 years (Boulos and Fahmy, 2007).

Subsequent floristic studies of the Egyptian grasses have been made by Täckholm (1941), Täckholm (1956, 1974), Ibrahim and Kabuye (1998), Cope (2005), and Boulos (2005). Cope and Hosni (1991) gave a comprehensive key to the Egyptian grasses, and the first key using vegetative features was done by Hosni and Ibrahim (2004). Recently, Peterson et al. (2020) presented a key to identify 284 Egyptian native and naturalized grass taxa in 103 genera belonging to 22 tribes and seven subfamilies. The key is fundamentally based on the floral features of the inflorescence and spikelet.

Stipagrostis Nees is placed within the Subfamily Aristidoideae Caro, Tribe: Aristideae C.E. Hubb. Systematically, Henrard (1929 – 1932) provided the only global systematic revision of the subfamily Aristidoideae, recognizing a single genus, *Aristida* L., with seven sections, 330 species, and 114 varieties, he admitted that sects. Schistachne (Fig. & De Not) Henrard and Stipagrostis (Nees) Trin. & Rupr. are characterized by different leaf anatomy from the other five sections, but he kept both in Aristida, moreover, concerning the heterogeneity of the sect. Streptachne (R. Br.) Domin, herecognized it for significant reasons but suggested merging it with sect. Chaetaria P. Beauv.) Trin.(= sect. Aristida). Later, De Winter (1965) recognized three sections: Anomala de Winter, Schistachne, and Stipagrostis, and he admitted that Schistachne and Stipagrostis should be united but retained both sections for taxonomic purposes.

De Winter (1965), Bourreil (1967), Brown (1977), and Balkwill et al. (2011) reported 50 species of

Stipagrostis; the Plant List Database (TPL) (2013), POWO (2024), and Soreng et al. (2022) recorded 56 species; Trobicos (2024) documented 69 species; GBIF (2024) recorded 72 species; and WFO (2024) recorded 70 species.

The Egyptian *Stipagrostis* treated only in general floristic studies, e.g., (Täckholm 1974, 1956; El-Hadidi and Fayed 1994, 1995; Boulos 2005, 2009; Ibrahim et al. 2016; Peterson et al. 2020), which lack proper synonymy lists, total type citations, and complete illustrations. The species number of *Stipagrostis* stated in Egypt has increased over time; Forsskål (1775) recognized only one species in Egypt (*Aristida lanata* Forssk). Raffeneau-Delile (1826) listed two further species in Egypt: *Aristida ciliata* Desf. and *Aristida obtusa* Delile. Boissier (1884) added nine species to Delile΄ s list. Then, Muschler (1912) recorded 11 species, while Ramis (1929) also listed 11 species, Täckholm (1974) reported 21 taxa (15 species and six varieties) of *Stipagrostis*, while Boulos (2005) described only 19 taxa (14 species and five varieties). Recently, Ibrahim et al. (2016) and Peterson et al. (2020) reported 14 species of *Stipagrostis* in the flora of Egypt. The distribution of the genus *Stipagrostis* is limited to the drier territories of Africa and the Middle East to Central Asia and western China (Cerros-Tlatilpa et al. 2011).

This study aimed to investigate the anatomical structure of stem and leaves of the taxa belonging to the genus *Stipagrostis* in Egypt for the first time, to decide on the significance of anatomical characters for delimitation of the studied taxa, and to use numerical taxonomy; UPGMA Cluster Analysis and PCA to understand better the relationships between taxa of the genus, and to identify characters that can be used to discriminate among taxa based on vegetative and floral characters.

MATERIALS AND METHODS

Stem and leaf anatomy

The present study is mainly based on herbarium materials kept at the following herbaria: CAI, CAIM, ASTU, ALEX, SHG, and K, in addition to fresh materials collected from different localities representing the various habitats of *Stipagrostis* in Egypt (Table 1). The acronyms for the herbaria used in this study are based on the continuously updated database by Thiers (2024). Fully mature branches were chosen. Fresh materials were fixed in formalin glacial acetic acid (FAA) and 70% Ethyl alcohol. The dried herbarium specimens were first softened usually by glycerin and ethyl alcohol.

Both the fresh and softened herbarium samples, preserved in 70% EtOH, were dehydrated with ethyl alcohol solutions of increasing strength. The lower third part of the stems and the middle third of the leaves have been sectioned at 20-30 µm using a Leitz 1512 microtome. After staining with safranin for 30 minutes, the tissue was rinsed with 50%, 70%, and 95% EtOH, respectively, for 3-5 minutes each. Light green (1%) stains plant tissues intensely in a very short time (30 sec.) (Gurr, 1965). The tissue was rinsed with 95%, 100% EtOH, and 99% xylene for 3-5 min each. The tissue was stabilized by a mounting medium (Canada Balsam) and covered by a slide cover. The prepared slides were dried in a hot air oven at 55°C for 48 hours to ensure proper fixation . Ten to fifteen slides were prepared for each taxon. Sections were examined, and Photomicrographs were taken with a compound microscope (Olympus BX51) at the Laboratory of Mycology, Botany and Microbiology Department, Faculty of Science, Sohag University. Terminology, according to De winter (1965), Metcalfe (1960), and Dogan (1982), with some modifications by authors, were used to describe leaf and stem anatomy. The stem transverse section of *S. vulnerans* and *S. paradisea* were very bad and dissected, so we did not present them in this study. We used them only to obtain the features used here.

Numerical analysis

The micromorphological, and anatomical data set matrices were subjected to Multivariate Statistical Package software (MVSP version 3.2) to calculate the Euclidean distance and construct the tree for cluster analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Average) according to Kovach (2007). The principal component analysis (PCA) for each data set, as well as the combined data, were performed using PAST (Paleontological Statistics version 3.15), according to Hammer (2001). A total of 58 characters were measured on each specimen, comprising 55 qualitative and three quantitative, 33 binary, and 25 multistate characters. In this analysis, species or subspecies constituted the OTU. The measurements for all specimens of a taxon were averaged into one OTU score for each character. The number of individuals of each taxon used in this study ranged from two to 32 because herbarium specimens cannot be considered a random sample of the species. For certain taxa, Egyptian materials were not accessible and specimens from other adjacent countries were utilized (OTU 5, 10, 12) (Table 2); for the complete data matrix, see Table 5.

Table 1. List of *Stipagrostis* specimens used for anatomical investigation.

No.	Taxon	Collector	Place of collection and herbarium	Date of collection
1	*S. ciliate* (Desf.) De Winter	Selim Zedan	Egypt, Sinai, Wadi Feran, (ALEX).	16.04.2008
2	*S. lanata* (Forssk.) De Winter	N. D. Simpson	Egypt, Sandy Palm Grove, Baltim, (K).	25.06.1927
3	*S. scoparia* (Trin. & Rupr.) De Winter	N. Tadmor	Egypt, N. Sanai, 23 km N. E. of Kantara rumami, sand dunes, (K).	28.12.1970
4	*S. vulnerans* (Trin. & Rupr.) De Winter	M. Abd El Ghani	Egypt, Bahariya Oasis: El-Heiz, sandy soil, (K).	27.08.1980
5	S. *drarii* (Täckh.) De Winter	J. S. Collenette	Saudi Arabia, 65 km of Tym-Madinah Road, (K).	12.03.1986
6	*S. raddiana* (Savi) De Winter	F. Saad	Egypt, Gebel Elba: W. Mitikwan, northern part, (K).	05.03.1963
7	*S. acutiflora* (Trin. & Rupr.) De Winter	V. Sobat	Saudi Arabia, Shabwa area. Dune binder in the Ramlat Sabtein, top of a dune forest, (K).	05.02.1952
8	*S. plumosa* var. *seminuda* Trin. & Rupr.	T. A. Cope, I. A. El-Garf & A. Amer	Egypt, Sinai, Between Wadi Feiran and Wadi Wirga, (K).	09.04.1990
9	*S. plumosa* var. *brachypoda* (Tausch) Trin. & Rupr.	Y. Abdel Aziz	Egypt, Cairo-Alex desert road, (SHG).	03.05.2020
10	*S. multinerva* H. Scholz	H. Akhani	Iran, Est du Dasht-e- Lut ā l'étude des grandes dunes, 59̊ 39 E, 30̊ 55 N, (K).	14.05.1972
11	*S. hirtigluma* (Steud. ex Trin. & Rupr.) De Winter	Y. Abdel Aziz	Egypt, Cairo-Alex desert road, (SHG).	03.05.2020
12	*S. uniplumis* (Licht.) De Winter	K. Smith	Saudi Arabia, 86 Km on the Mecca bypass, (K).	07.05.1982
13	*S. paradisea* (Edgew.) De Winter	N. D. Simpson	Egypt, North Glala, (K).	02.04.1924
14	*S. obtuse* (Delile) Nees	G. Täckholm	Egypt, Wadi El Rakhama between Maadi and Suez, (CAI).	15.04.1926

Table 2. List of OUTs for the *Stipagrostis* species used for numerical studies.

No	Taxon	Origin	NO. of individuals	Boisser (1884)	Boulos (2005)
1	*S. ciliata* (Desf.) De Winter	Egypt	18	Sect. Stipagrostis	Sect. Schistachne
2	*S. lanata* (Forssk.) De Winter	Egypt, Palestine	15	Sect. Stipagrostis	Sect. Stipagrostis
3	*S. scoparia* (Trin. & Rupr.) De Winter	Egypt, Palestine	7	-	Sect. Stipagrostis
4	*S. vulnerans* (Trin. & Rupr.) De Winter	Egypt	4	-	Sect. Stipagrostis
5	*S. drarii* (Tackh.) De Winter	Saudi Arabia	5	-	Sect. Stipagrostis
6	*S. raddiana* (Savi) De Winter	Egypt, Palestine, Jordan	8	-	Sect. Stipagrostis
7	*S. acutiflora* (Trin. & Rupr.) De Winter	Egypt, Saudi Arabia, United Arab Emirates	16	Sect. Stipagrostis	Sect. Stipagrostis
8	*S. plumosa* (L.) Munro ex T. Anderson var. *seminuda* (Trin. & Rupr.) H. Scholz	Egypt	32	Sect. Stipagrostis	Sect. Stipagrostis
9	*S. plumosa* (L.) Munro ex T. Anderson var. *brachypoda* (Tausch) Bor	Egypt, El-Bahrein	26	Sect. Stipagrostis	Sect. Stipagrostis
10	*S. multinerva* H. Scholz	Iran, Oman	2	-	Sect. Stipagrostis
11	S. hirtigluma (Steud. ex Trin. & Rupr.) De Winter	Egypt	4	Sect. Stipagrostis	Sect. Stipagrostis
12	*S. uniplumis* (Licht.) De Winter	Saudi Arabia, Oman	5	Sect. Stipagrostis	Sect. Stipagrostis
13	*S. paradisea* (Edgew.) De Winter	Egypt	1	-	Sect. Stipagrostis
14	*S. obtuse* (Delile) Nees	Egypt	2	Sect. Stipagrostis	Sect. Stipagrostis

RESULTS

Stem and leaf anatomical characters were valuable in distinguishing among taxa of the genus *Stipagrostis* in Egypt. Tables 3, 4, and Plates I, II present anatomical characters observed using light microscopy.

Stem anatomy

Stem shape: The stem cross-section shape of the *Stipagrostis* is ovate, circular, rectangular, broadly rectangular, or triangle. However, it is ovate in *S. ciliata*, *S. hirtigluma* and *S. obtusa* (Plate I: 1a, 10a, 12a), circular in *S. lanata*, *S. raddiana*, *S. acutiflora*, *S. plumosa* var *seminuda*, *S. plumosa* var *brachypoda* and *S. uniplumis* (Plate I: 2a, 5a, 6a, 7a, 8a, 11a), rectangular in *S. drarii* (Plate I: 4a), broadly rectangular in *S. scoparia* (Plate I: 3) and triangle in *S. multinerva* (Plate I: 9a).

Stem outer boundary: The outer boundary of the *Stipagrostis* stem varies, appearing straight in some species and waved in others. It is straight in *S. ciliata*, *S. multinerva*, and *S. hirtigluma* (Plate I: 1a, 9a, 10a) while being waved in the rest of the studied taxa (Table 3).

Sclerenchyma and Vascular Bundles

Sclerenchyma thickness: Sclerenchyma cells under the epidermis varied in thickness from 30 to 88 µm. *S. ciliata* and *S. uniplumis* have the thickest sclerenchyma layers (Plate I: 1b and 11b), whereas *S. plumosa* var. *brachypoda* and *S. multinerva* exhibit the thinnest layers (Plate I: 8b and 9b), (Table 3).

Vascular system: The vascular bundles of the *Stipagrostis* stem are scattered or arranged in two or three lines. It is two lines in *S. ciliata*, *S. raddiana*, *S. plumosa* var. *seminuda*, *S. hirtigluma,* and *S. obtusa*

Table 3. Summary of the stem differential anatomical characters of *Stipagrostis* taxa.

No.	Taxa	Stem shape in T.S.	Diameter of cross section in (μm)	Sclerenchyma thickness (μm)	Outer frame	V. B. line number	Length of major V.B. (μm)	Length of minor V.B (μm)	Assimilatory tissue
1	*S. ciliata*	Ovate	783× 633	75	Straight	2	90.9	50	Narrow
2	*S. lanata*	Circular	954.5	50	Waved	Scattered	136.36	45.45	Wide
3	*S. scoparia*	Broadly rectangular	895× 633	42.5	Waved	Scattered	113.36	22.72	Narrow
4	*S. drarii*	Rectangular	1000×833	55	Waved	Scattered	138	45	Wide
5	*S. raddiana*	Circular	659	50	Waved	2	75	25	Wide
6	*S. acutiflora*	Circular	727	42.5	Waved	Scattered	115	23	Wide
7	*S. plumosa* var. *seminuda*	Circular	568	37.5	Waved	2	77.27	31.81	Wide
8	*S. plumosa* var. *brachypoda*	Circular	613.5	30	Waved	3	109	36.36	Wide
9	*S. multinerva*	Triangle	522.7×477	30	Straight	Scattered	107.5	68.18	Wide
10	*S. hirtigluma*	Ovate	583×508	35	Straight	2	87.5	42.5	Narrow
11	*S. uniplumis*	Circular	625	87.5	Waved	3	125	20	Wide
12	*S. obtusa*	Ovate	458×583	50	Waved	2	75	35	Narrow

Table 4. Summary of the leaf differential anatomical characters of *Stipagrostis* taxa, ab = abaxiale, ad = adaxiale, Vbs = Vascular bundles.

No.	Taxa	Shape of leaf blade in T. S	Vascular bundle shape			Vascular bundle number			Outer sheath	
			1˚	2˚	3˚	1˚	2˚	3˚	Shape	extent
1	*S. ciliata*	Round	Elliptical	Round	Round	5	8	2	Oblong	Incomplete ab/ad 1˚Vbs, ab/2˚Vbs
2	*S. lanata*	Elliptical	Elliptical	Round	Round	5	2	4	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
3	*S. scoparia*	Round	Elliptical	Round	Absent	7	8	0	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
4	*S. vulnerans*	U-shaped	Elliptical	Round	Round	7	6	2	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
5	*S. drarii*	Round	Elliptical	Round	Absent	5	6	0	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
6	*S. raddiana*	Round	Elliptical	Round	Round	5	8	2	Oblong	Incomplete- ab/ad 1 Vbs, ab/2˚Vbs
7	*S. acutiflora*	Elliptical	Elliptical	Round	Round	5	4	2	Oblong	Incomplete- ab/ad 1˚Vbs, ad/2˚Vbs
8	*S. plumosa* var. *seminuda*	Elliptical	Absent	Elliptical	Round	0	3	6	Elliptical	Incomplete- ab/2˚Vbs
9	*S. plumosa* var. *brachypoda*	Round	Elliptical	Round	Round	5	4	2	Oblong	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
10	*S. multinerva*	U-shaped	Absent	Elliptical	Round	0	4	5	Elliptical	Incomplete- ab/2˚Vbs
11	*S. hirtigluma*	Round	Elliptical	Round	Round	5	7	2	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs
12	*S. uniplumis*	Round	Absent	Elliptical	Round	0	5	6	Elliptical	Incomplete- ab/2˚Vbs
13	*S. paradisea*	Round	Elliptical	Elliptical	Round	2	3	7	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚,3˚ Vbs
14	*S. obtusa*	Round	Elliptical	Round	Round	3	4	2	Elliptical	Incomplete- ab/ad 1˚Vbs, ab/2˚Vbs

Table 4 (**Continued**). OS = outer bundle sheath, IS = inner bundle sheath

Character	Inner sheath		Sclerenchyma (adaxial)		Sclerenchyma (abaxial)		Types of bulliform cell
Taxa	Extent	Structure	Strands	Girders	Strands	Girders	
S. ciliata	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Trapezoidal	2˚,1˚Vbs	T-shape	Irregular
S. lanata	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	T-shape	Irregular
S. scoparia	Complete	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	Trapezoidal	Irregular
S. vulnerans	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	Below vbs	Regular
S. drarii	Complete	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	Trapezoidal	Irregular
S. raddiana	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Trapezoidal	2˚,1˚Vbs	Below vbs	Irregular
S. acutiflora	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Trapezoidal	2˚,1˚Vbs	Arched	Irregular
S. plumosa var. *seminuda*	Complete	OS > IS	Absent	-	2˚Vbs	Trabezoidal	Regular
S. plumosa var. *brachypoda*	Complete	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	Trabezoidal	Irregular
S. multinerva	Incomplete-ab 2˚Vbs	OS > IS	Absent	-	2˚Vbs	Trabezoidal	Fan- shaped
S. hirtigluma	Incomplete-ab 1˚Vbs	OS > IS	1˚Vbs	Arched	2˚,1˚Vbs	Trapezoidal	Fan- shaped
S. uniplumis	Incomplete-ab 2˚Vbs	OS > IS	Absent	-	2˚Vbs	Trabezoidal	Irregular
S. paradisea	Complete	OS > IS	2˚,1˚Vbs	Arched	3˚, 2˚,1˚Vbs	Trapezoidal	Irregular
S. obtusa	Complete	OS > IS	Absent	-	2˚,1˚Vbs	Trabezoidal	Irregular

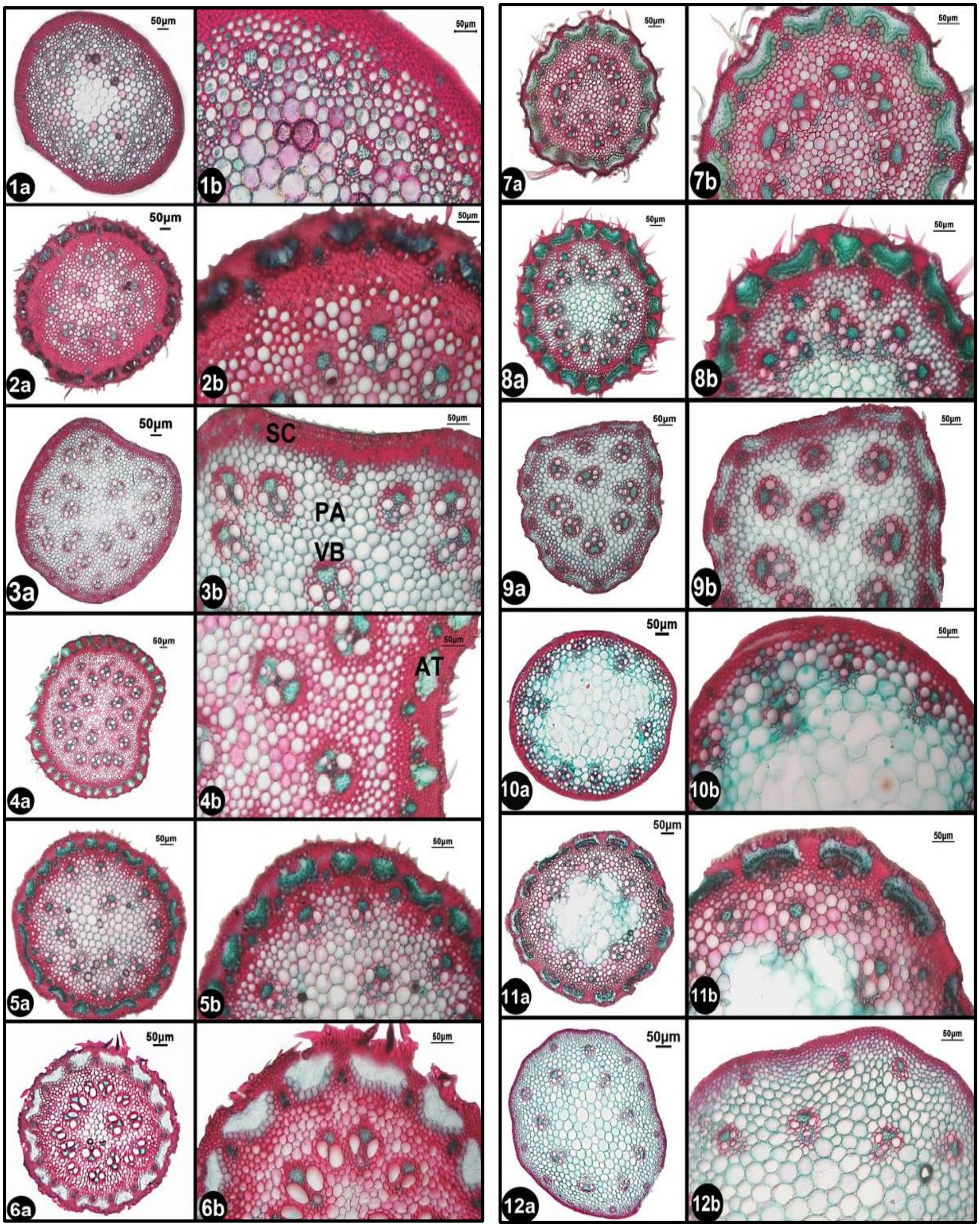

Plate I. Photomicrographs of transverse stem sections: a) entire transverse stem section; b) stem enlargement showing internal structure; Scale bar = 50 µm; VB = Vascular bundle, PA = Parenchyma, SC = Sclerenchyma, AT = Assimilatory tissue. 1) *S. ciliata*, 2) *S. lanata*, 3) *S. scoparia*, 4) *S. Drarii,* 5) *S. raddiana*, 6) *S. Acutiflora*, 7) *S. plumosa* var. *seminuda*, 8) *S. plumosa* var. *Brachypoda,* 9) *S. multinerva*, 10) *S. hirtigluma*, 11) *S. uniplumis*, 12) *S. obtuse*.

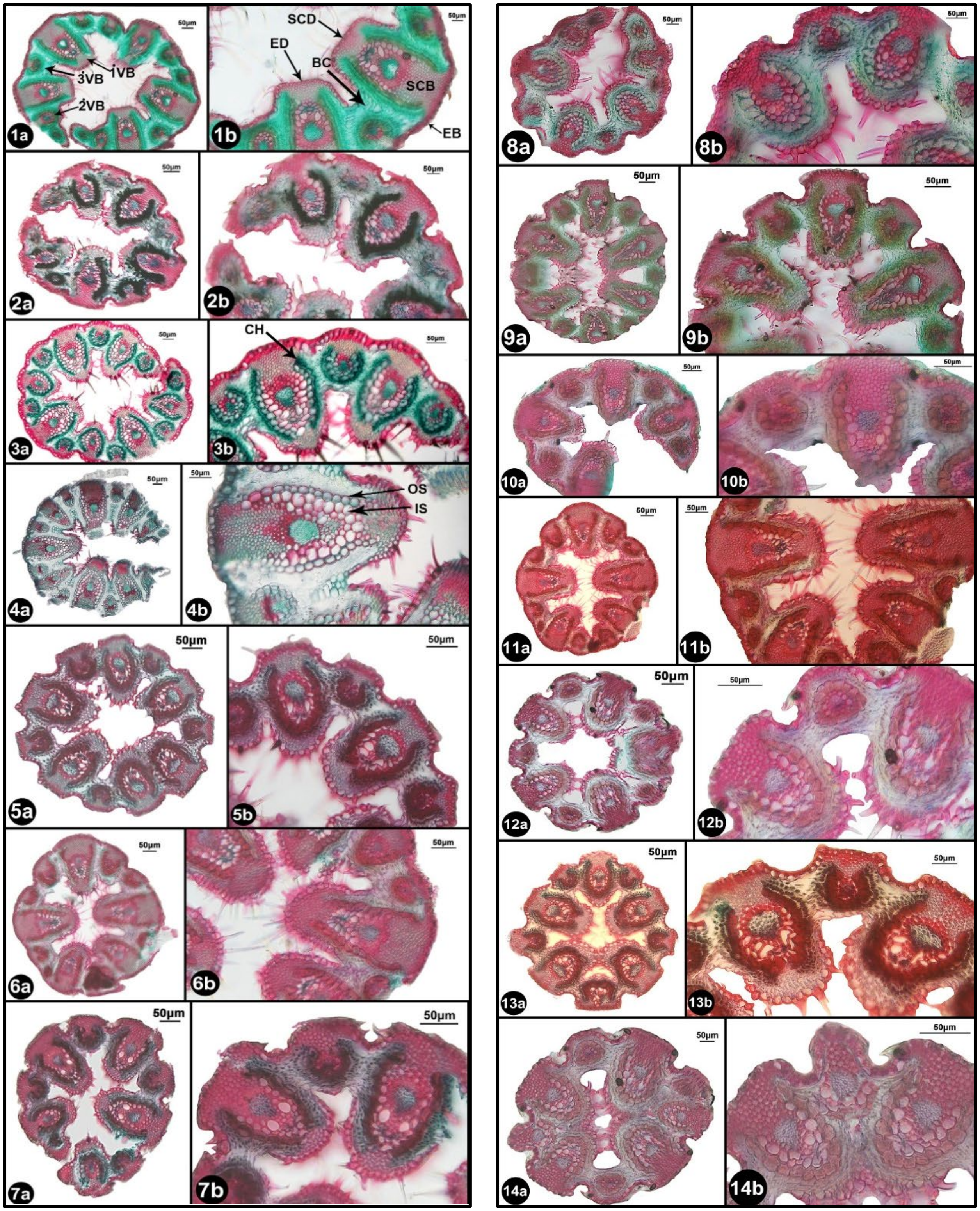

Plate II. Photomicrographs of transverse leaf sections: a) entire transverse leaf section; b) leaf enlargement showing internal structure; Scale bar = 50 µm; 1°VB = primary vascular bundle, 2°VB = secondary vascular bundle, 3°VB = tertiary vascular bundle, ED = adaxial epidermis, EB = abaxial epidermis, BC = bulliform cells, SCD = sclerenchyma adaxile, SCB = sclerenchyma abaxile, CH = Chlorechyma, OS = outer bundle sheath, IS = inner bundle sheath. 1) *S. ciliata*, 2) *S. lanata*, 3) *S. scoparia*, 4) *S. vulnerans*, 5) *S. Drarii,* 6) *S. raddiana*, 7) *S. Acutiflora*, 8) *S. plumosa* var. *seminuda*, 9) *S. plumosa* var. *brachypoda,* 10) *S. multinerva,* 11) *S. hirtigluma,* 12) *S. uniplumis*, 13) *S. pradisea*, 15) *S. obtuse.*

(Plate I: 1a, 5a, 7a, 10a, 12a); three in *S. plumosa* var. *brachypoda*, and *S. uniplumis* (Plate I: 8a, 11a) and scattered in *S. lanata*, *S. scoparia*, *S. drarii*, *S. acutiflora* and *S. multinerva* (Plate I: 2a, 3a, 4a, 6a, 9a). The length of the significant vascular bundle ranged from 75 to 138 µm, while the length of minor vascular bundles from 20 to 68.18 µm (Table 3).

Assimilatory tissue: The assimilatory tissue of the *Stipagrostis* stem varies from narrow to wide. However, it is narrow in *S. ciliata*, *S. scoparia*, *S. hirtigluma*, and *S. obtusa* (Plate I: 1b, 3b, 10b, 12b) while wide in the rest of the studied taxa (Table 3).

Leaf anatomy

The shape of the leaf blade

It is easily seen from the leaf transverse sections that the shape of the *Stipagrostis* leaf blade section varied from rounded, elliptical to U-shaped. However, it is elliptical in *S. lanata*, *S. acutiflora*, and *S. plumosa* var. *seminuda* (Plate II: 2a, 7a and 8a), respectively; U-shaped in *S. vulnerans* and *S. multinerva* (Plate II: 4a, 10a) respectively, while it is rounded in the rest of the studied taxa (Table 4).

Adaxial and Abaxial Furrows

The furrows of both adaxial and abaxial leaf surfaces are the same in all taxa; however, they are deep in adaxial surfaces and shallow in abaxial surfaces (Plate II).

Vascular bundles

In *Stipagrostis* three types of vascular bundles were recognized: primary, secondary, and tertiary, which vary in shape from elliptical to round. The primary vascular bundles are elliptical in all taxa under investigation except for *S. plumosa* var*. seminuda, S. multinerva,* and *S. uniplumis,* which lack primary vascular bundles (Plate II: 8, 10, 12). The secondary vascular bundles vary from round to elliptical. However, they are rounded in all taxa under investigation except for *S. plumosa* var*. seminuda, S. multinerva, S. uniplumis,* and *S. paradisea,* which have elliptical secondary vascular bundles shape (Plate II: 8, 10, 12, 13). The tertiary vascular bundles are rounded in taxa under study except for *S. scoparia* and *S. drarii,* where they are absent (Plate II: 3, 5). The outer bundle sheath shape varies from oblong to elliptical. However, it is oblong in *S. ciliata, S. raddiana, S. acutiflora,* and *S. plumosa* var*. brachypoda* (Plate II: 1b, 6b, 7b, 9b), while it is elliptical in the rest of the studied taxa. The extent of outer bundle sheath is incomplete in abaxial/adaxial primary vascular bundles as well as in abaxial secondary vascular bundles in *S. ciliata, S. lanata, S. scoparia, S. vulnerans, S. drarii*, *S. raddiana, S. acutiflora*, *S. plumosa* var*. brachypoda*, *S. hirtigluma* and *S. obtusa*, (Plate II: 1b, 2b, 3b, 4b, 5b, 6b, 7b, 9b, 11b, 14b), while it is incomplete in abaxial of secondary vascular bundles only in *S. plumosa* var*. seminuda, S. multinerva* and *S. uniplumis* (Plate II: 8b, 10b, 12b), moreover it is incomplete in abaxial/adaxial primary vascular bundles as well as in abaxial of secondary and tertiary vascular bundles in *S. paradisea* (Plate II: 13b)*.* The inner bundle sheath extent is complete around all vascular bundles in *S. plumosa* var. *seminuda, S. plumosa* var. *brachypoda, S. paradisea,* and *S. obtusa* (Plate II: 8b, 9b, 13b, 14b), while it is incomplete around primary vascular bundles only in *S. ciliata, S. lanata, S. scoparia, S. vulnerans, S. drarii*, *S. raddiana, S. acutiflora* and *S. hirtigluma* (Plate II: 1b, 2b, 3b, 4b, 5b, 6b, 7b, 11b), moreover *S. multinerva* and *S. uniplumis* characterized by incomplete inner bundle sheath around secondary vascular bundles only (Plate II: 10b, 12b) respectively.

Sclerenchymatous cells

The transverse sections of leaf blades in *Stipagrostis* demonstrated that all the taxa under study except for *S. plumosa* var. *seminuda, S. multinerva,* and *S. uniplumis* have adaxial sclerenchyma girder on the primary vascular bundles, which vary in shape from trapezoidal to arched. However, it is trapezoidal in *S. ciliata, S. raddiana* and *S. acutiflora* (Plate II: 1b, 6b, 7b), while it is arched in *S. lanata, S., scoparia, S. vulnerans, S. drarii, S. hirtigluma* and *S. paradisea* (Plate II: 2b, 3b, 4b, 5b, 11b, 13b) respectively. The trapezoidal abaxial sclerenchyma girder is found only on secondary vascular bundles in *S. plumosa* var. *seminuda, S. multinerva,* and *S. uniplumis* (Plate II: 8b, 10b, 12b). The sclerenchyma girder varies from trapezoidal to T-shaped on both primary and secondary vascular bundles. However, it is trapezoidal in *S. scoparia, S. drarii, S. plumosa* var. *brachypoda, S. hirtigluma,* and *S. obtusa* (Plate II: 3b, 5b, 9b, 11b, 14b) respectively, whereas it is T-shaped in *S. ciliata* and *S. lanata* (Plate II: 1b, 2b).

Chlorenchyma cells

Stipagrostis leaves have two bundle sheaths enveloped by radiate tabular-shaped chlorenchyma cells, and the outer sheath has centripetally positioned chloroplasts.

Bulliform cells

Transverse sections of the *Stipagrostis* leaf blades taxa under investigation show that the bulliform cells are located on the adaxial surfaces of leaves. They vary in shape from regular and irregular to fan shaped. However, they are regular in *S. vulnerans* and *S. plumosa* var. *seminuda* (Plate II: 4b and 8b), fan-shaped in *S. multinerva* and *S. hirtigluma* (Plate II: 10b, 11b), and irregular in the rest of the studied taxa (Plate II, Table 4).

Numerical studies

Cluster analysis

The phenogram calculated by UPGMA (Figure 1) shows the highest co-phenetic correlation Euclidean (0.9622), which indicates a good fit between the phenogram and the distance matrix. The phenogram was obtained using 58 morphological, micromorphological, and anatomical characters (Table 5). At the Euclidean distance of 8.2, two major clusters were split: C1 and C2. The first cluster, C1, includes only one species: *S. ciliata*, sect. Schistachne. The second cluster, C2 represents sect. Stipagrostis split into three groups: A, B, and C, with Euclidean distance of 7.5. Group (A) includes *S. scoparia*, *S. vulnreans,* and *S. drarii,* which are grouped in one clade. Whereas, at Euclidean distance of 6.8, group (B) divides into two subgroups b1 and b2: first subgroup (b1) includes *S. hirtigluma*, *S. paradisea*, *S. obtusa*, and *S. uniplumis*. In contrast, the second subgroup (b2) comprises *S. multinerva*, and *S. plumosa* var. *seminoda*. Group (C) involves *S. lanata*, *S. plumosa* var. *brachypoda,* which are grouped in one clade at Euclidean distance of 6.00, and *S. raddiana*, *S. acutiflora* where clustered at Euclidean distance of 6.2.

Principle Component Analysis PCA

The PCA provided numerical values corresponding to the morphological, micromorphological, and anatomical attributes used in our taxonomic analysis. The plot of 14 OTUs on the first two principal component axes, as shown in (Figure 2), explains 44.51% of the total observed variation. Segregation between the three groups was clear on the first axis (25.559% of the total variation, Figure 2). 1) Group of *S. hirtigluma*, *S. paradisea*, *S. obtusa*, *S. uniplumis*, *S. multinerva*, and *S. plumosa* var. *seminoda*. 2) Group of *S. scoparia*, *S. vulnreans* and *S. drarii*. 3) group of *S. ciliata*. On the second axis (18.951% of the total variation, Figure 2) revealed a split of *S. lanata*, *S. plumosa* var. *brachypoda*, *S. raddiana,* and *S. acutiflora*.

Key to the studied taxa based on leaf and stem anatomical characters

1a. Shape of leaf blade in T.S. is U-shaped ……………………… 2
1b. Shape of the leaf blade in T.S. is elliptical or rounded ..3
2a. Sclerenchyma adaxial girder absent …………… *S. obtusa*
2b. Sclerenchyma adaxial girder present…………………….. ……………………………………… *S. plumosa* var. *brachypoda*
3a. Shape of the leaf blade in T.S. is elliptical ………………… 4
3b. Shape of the leaf blade in T.S. is round 5
4a. Outline shape of the stem cross-section is a triangle .... *S. multinerva*
4b. Outline shape of the stem cross section is circular .... *S. plumosa* var. *seminuda*
5a. Secondary vascular bundle of leaf T.S. is elliptical 6
5b. Secondary vascular bundle of leaf T.S. is round 7
6a. Primary vascular bundle of leaf T.S. is elliptical .... *S. paradisea*
6b. Primary vascular bundle of leaf T.S. is absent .... *S. uniplmis*
7a. Outer sheath of leaf vascular bundles is elliptical 8
7b. Outer sheath of leaf vascular bundles is oblong 12
8a. Inner sheath of leaf vascular bundles is complete in all vascular bundles 9
8b. Inner sheath is incomplete in abaxial primary vascular bundles 10
9a. Outline shape of the stem cross-section is rectangular .... *S. drarii*
9b. Outline shape of the stem cross-section is broadly rectangular .... *S. scoparia*
10a. Shape of bulliform is Fan-shaped *S. hirtigluma*
10b. Shape of bulliform is regular or irregular 11
11a. Shape of bulliform is regular .... *S. vulnerans*
11b. Shape of bulliform is regular or irregular ...... *S. lanata*
12a. Outline shape of the stem cross-section is ovate .... *S. ciliata*
12b. Outline shape of the stem cross section is circular ..13
13a. Vascular bundles line number of the stem is two .... *S. raddiana*
13b. Vascular bundles of the stem are scattered .... *S. acutiflora*

DISCUSSION

The anatomical features of Poaceae were first utilized for taxonomic purposes by Duval-Jouve (1875), who declared that the position, presence or absence, and type of the bulliform cells in the leaves could be considered significant diagnostic characteristics. Schwendener (1890) highlighted the presence of sclerenchyma between the vascular bundles and the upper or lower epidermises to be of systematic importance. Moreover, Vukolov (1929) showed the diagrammatical arrangement of sclerenchyma around the vascular bundles. In 1960, Metcalfe examined about 345 genera of Poaceae and found diagnostic microscopical characters as the shape

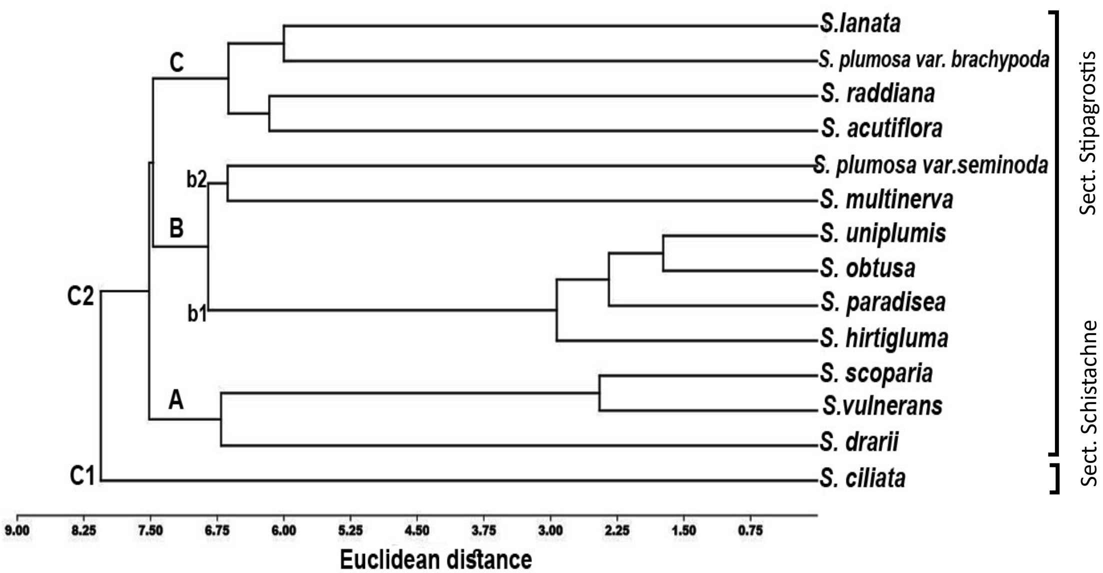

Figure 1. UPGMA dendrogram of the 14 taxa of *Stipagrostis* based on 58 morphological, micromorphological, and anatomical characters.

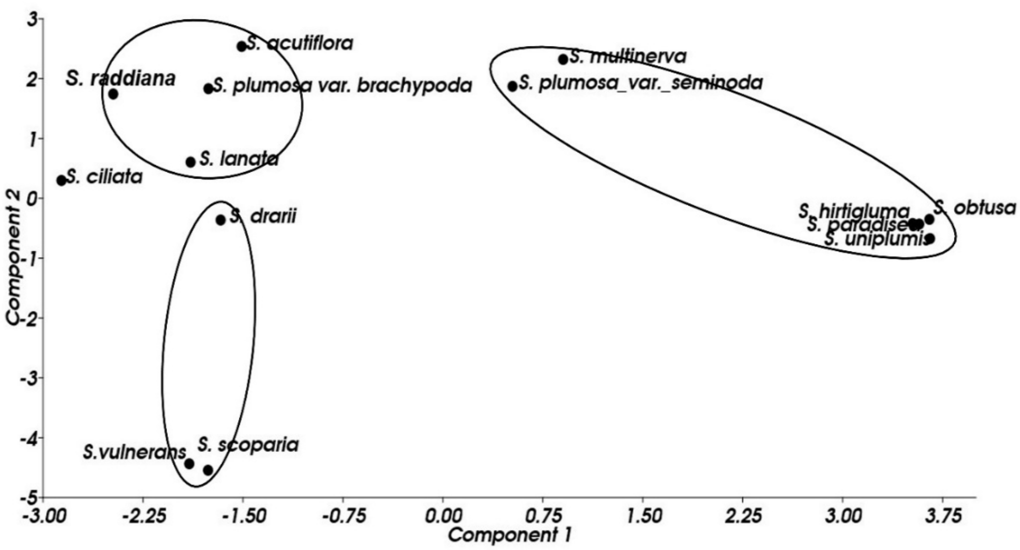

Figure 2. Scatter plot of the 14 OTUs of *Stipagrostis* plotted against the first principal component by the second principal component based on 58 morphological, micromorphological, and anatomical characters.

of girders, the strands, and the stomata types based on subsidiary cells (Mavi 2012).

The outline shape of the stem is of a high taxonomic value in the segregation of *S. scoparia*, *S. drarii*, and *S. multinerva*, *and* these taxa are characterized by broadly rectangular, rectangular, and triangle stem outline shapes, respectively (Plate I: 3a, 4a, 9a). Moreover, the outline shape distinguished the most morphologically similar species *S. drarii,* from *S. raddiana*, as it is rectangular in *S. drarii,* and circular in *S. raddiana*. Also, *S. multinerva* is segregated from *S. plumosa*, as the stem outline shape is a triangle in *S. multinerva* and circular in *S. plumosa*. Additionally, the stem outline shape is ovate in *S. hirtigluma* while circular in *S. uniplumis*. Stover (1951) classified the family members Poaceae into three groups according to the arrangement of their internodular vascular bundles; these vascular bundles are arranged in one layer, two layers, or scattered. Culm transverse sections of the tribe triaceae (Family: Poaceae) demonstrate that the vascular bundles are arranged in 2 or 3 circular rings (Mavi 2012).

Table 5. Data matrix based on 58 macro, micromorphological, and anatomical character states of stem, leaf, and nutlet.

Characters	Character states	Taxa													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. stem	Herbaceous	1	1	0	0	1	1	1	1	1	1	1	1	1	1
	Suffrutescent	0	0	1	1	0	0	0	0	0	0	0	0	0	0
2. leaf curvature	Curved	1	0	0	0	1	0	0	0	0	0	0	0	0	0
	Straight	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Curved or straight	0	1	1	1	0	1	1	1	1	0	1	0	1	1
3. Basilar tufts	Present	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	0	1	1	1	1	1	1	1	1	1	1	1	1	1
4. lower leaf surface	Glabrous	0	0	1	1	0	0	0	1	1	0	0	1	0	0
	Scaperous	1	0	0	0	1	0	0	0	0	1	1	1	1	1
	Pubscent	0	1	0	0	0	1	1	0	0	0	0	0	0	0
5. Upper leaf surface	Hairy	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	Scaperous	0	0	0	0	0	0	0	1	1	0	0	1	0	0
	Pubscent	1	0	0	0	0	0	0	0	0	0	0	0	0	0
6. leaf sheath	Lower wooly	0	0	0	0	0	1	0	1	1	0	0	0	0	0
	Glaberous	0	1	1	1	1	0	0	0	0	1	1	0	1	1
	Pubscent	1	0	0	0	0	0	1	0	0	0	0	0	0	0
7. Node texture	Glaberous	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	Bearded	1	0	0	0	0	0	0	0	0	0	0	0	0	0
8. internode texture	Glabrous	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	Wooly	1	0	1	1	0	0	0	0	0	0	1	1	1	1
	Lower wooly	0	1	0	0	1	1	1	0	0	1	0	0	0	0
9. panicle	Tightened	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	lax	1	1	0	0	0	1	1	1	1	1	1	1	1	1
10. Central awn feathers	Feathery to the base	0	0	1	0	1	0	0	0	0	0	0	0	1	0
	Feathery with a naked tip	0	0	0	1	0	1	0	0	0	0	1	1	0	0
	Feathery with naked last part and tip	1	1	0	0	0	0	1	1	1	1	0	0	0	1
11. lateral awn	Feathery	0	1	1	1	0	0	0	0	0	0	0	0	0	0
	Naked	1	0	0	0	1	1	1	1	1	1	1	1	1	1
12. lower glume nerve number	Three	1	0	0	0	1	1	0	1	1	0	1	1	1	1
	From 1 to 3	0	1	0	1	0	0	1	0	0	0	0	0	0	0
	More than 3	0	0	1	0	0	0	0	0	0	1	0	0	0	0
13. lower glume texture	Glabrous	1	0	1	1	0	1	0	0	0	0	0	0	0	0
	Hairy	0	1	0	0	1	0	1	1	1	1	1	1	1	1
14. Upper glume apex	Acute	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	Obtuse	1	0	0	0	0	0	0	0	0	0	0	0	0	0
15. Upper glume nerve number	Three	1	1	1	0	1	0	1	0	0	0	1	1	1	1
	From 1 to 3	0	0	0	1	0	1	0	1	1	0	0	0	0	0
	More than 3	0	0	0	0	0	0	0	0	0	1	0	0	0	0
16. Upper glume texture	Glabrous	1	0	1	1	1	1	0	1	0	0	1	1	1	1
	Hairy	0	1	0	0	0	0	1	0	1	1	0	0	0	0
17. Shape of long cell of abaxial leaf	Rectangular to oblong	1	0	1	1	1	1	1	1	0	1	1	1	1	1
	Oblong	0	1	0	0	0	0	0	0	1	0	0	0	0	0
18. Short cell of abaxial leaf	Present	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	Absent	1	1	1	1	1	1	1	1	0	0	1	1	1	1
19. Silica cell of abaxial leaf	Present	1	1	1	1	0	1	1	1	1	0	1	1	1	1
	Absent	0	0	0	0	1	0	0	0	0	1	0	0	0	0
20. Shape of silica cell of abaxial leaf	Rounded to square	1	1	0	0	0	0	1	0	0	0	0	0	0	0
	Rod -shape	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Rounded	0	0	0	0	0	1	0	0	1	0	1	1	1	1
	Rounded to rectangular	0	0	0	0	0	0	0	1	0	0	0	0	0	0
21. Prickle of abaxial leaf	Present	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	Absent	1	0	0	0	0	0	0	0	0	0	0	0	0	0
22. Macro-hairs of abaxial leaf	Present	0	1	0	0	0	1	0	0	1	0	0	0	0	0
	Absent	1	0	1	1	1	0	1	1	0	1	1	1	1	1
23. Stomata of abaxial leaf	Present	0	0	1	1	0	0	1	0	0	1	1	1	1	1
	Absent	1	1	0	0	1	1	0	1	1	0	0	0	0	0
24. Shape of long cell of adaxial leaf	Undifferentiated	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rectangular to oblong	0	1	0	0	1	1	1	0	1	1	1	1	1	1
	Oblong	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Rectangular	0	0	1	1	0	0	0	0	0	0	0	0	0	0
25. Wall of long cell of adaxial leaf	Undifferentiated	0	0	0	0	0	0	1	0	0	1	0	0	0	0
	Undulate	0	1	1	1	1	0	0	1	1	0	1	1	1	1
	Highly undulate	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Straight	1	0	0	0	0	0	0	0	0	0	0	0	0	0
26. Silica cell of adaxial leaf	Present	0	0	0	0	0	0	1	0	0	1	0	0	0	0
	Absent	1	1	1	1	1	1	0	1	1	0	1	1	1	1
27. The shape of the silica cell of the adaxial leaf	Rounded	0	0	0	0	0	0	1	0	0	1	1	1	1	1
28. The prickle of the adaxial leaf	Present	0	0	0	0	1	0	1	1	0	0	0	0	0	0
	Absent	1	1	1	1	0	1	0	0	1	1	1	1	1	1
29. Macro hairs of adaxial leaf	Present	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Absent	0	0	0	0	0	0	0	0	0	0	0	0	0	0

30. Stomata of adaxial leaf	Present	0	1	0	0	0	0	1	0	0	0	1	1	1	1
	Absent	1	0	1	1	1	1	0	1	1	1	0	0	0	0
31. The shape of the long cell of stem	Undifferentiated	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Rectangular to oblong	1	0	1	1	0	0	1	1	0	1	1	1	1	1
	Oblong	0	0	0	0	0	1	0	0	1	0	0	0	0	0
32. Wall of the long cell of stem	Undifferentiated	0	0	0	0	0	0	1	0	1	0	0	0	0	0
	Undulate	0	0	1	1	0	0	0	0	0	0	1	1	1	1
	Highly undulate	1	0	0	0	0	1	0	1	0	1	0	0	0	0
	Straight	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Present	1	0	0	0	0	1	1	0	0	0	0	0	0	0
33. Short cell of stem	Absent	0	1	1	1	1	0	0	1	1	1	1	1	1	1
	Present	1	0	1	1	1	1	1	1	0	1	1	1	1	1
34. Silica cell of stem	Absent	0	1	0	0	0	0	0	0	1	0	0	0	0	0
	Square	0	0	0	0	0	0	0	0	0	0	1	1	1	1
35. Shape of Silica cell of stem	Rounded to rectangular	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Rounded	1	0	1	1	1	1	1	0	1	1	0	0	0	0
36. Prickle of stem	Present	1	0	0	0	1	1	1	1	0	1	0	0	0	0
	Absent	0	1	1	1	0	0	0	0	1	0	1	1	1	1
37. Macro-hairs of stem	Present	0	1	0	0	1	1	1	1	1	1	0	0	0	0
	Absent	1	0	1	1	0	0	0	0	0	0	1	1	1	1
38. Stomata of stem	Present	0	0	0	0	0	1	1	0	0	0	1	1	1	1
	Absent	1	1	1	1	1	0	0	1	1	1	0	0	0	0
39. Nutlet shape	Narrowly deltoid	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lanceolate	0	1	0	0	1	0	1	1	1	1	1	1	1	1
	Oblong	0	0	1	1	0	1	0	0	0	0	0	0	0	0
40. Shape of an epidermal cell of nutlet	Rectangular to oblong	1	1	0	0	1	1	1	1	1	1	1	1	1	1
	Wrinkled	0	0	1	1	0	0	0	0	0	0	0	0	0	0
41. Anticlinal wall of epidermal cell of nutlet	Undulate	0	0	0	0	0	1	1	1	1	1	0	0	0	0
	Straight	0	0	0	0	1	0	0	0	0	0	1	1	1	1
	Highly undulate	1	1	1	1	0	0	0	0	0	0	0	0	0	0
42. Silica cell	Present	0	0	1	1	0	0	0	0	0	0	1	1	1	1
	Absent	1	1	0	0	1	1	1	1	1	1	0	0	0	0
43. Stem shape in T.S.	Ovate	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Circular	0	1	0	0	0	1	1	1	1	0	1	1	1	1
	Triangular	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Rectangular	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	Broadly rectangular	0	0	0	0	1	0	0	0	0	0	0	0	0	0
44. Outer frame of stem T.S	Waved	0	1	1	1	1	1	1	1	1	0	1	1	1	1
	Straight	1	0	0	0	0	0	0	0	0	1	0	0	0	0
45. Vascular bundle line number in stem T.S	Two	1	0	0	0	0	1	0	1	0	0	0	0	0	0
	Three	0	0	0	0	0	0	0	0	1	0	1	1	1	1
	Scattered	0	1	1	1	1	0	1	0	0	1	0	0	0	0
46. Assimilatory tissue	Narrow	1	0	1	1	0	0	0	0	0	0	0	0	0	0
	Wide	0	1	0	0	1	1	1	1	1	1	1	1	1	1
47. Shape of leaf blade in T.S	Round	1	1	1	1	1	1	1	0	0	0	1	1	1	1
	Elliptical	0	0	0	0	0	0	0	1	0	1	0	0	0	0
	U-shape	0	0	0	0	0	0	0	0	1	0	0	0	0	0
48. Tertiary V.B.s shape	Round	1	1	0	0	0	1	1	1	1	1	1	1	1	1
	Absent	0	0	1	1	1	0	0	0	0	0	0	0	0	0
49. Secondary V.B.s shape	Elliptical	0	0	0	0	0	0	0	1	0	1	1	1	1	1
	Round	1	1	1	1	1	1	1	0	1	0	0	0	0	0
50. Primary V.B.s shape	Absent	1	1	1	1	1	1	1	0	1	0	0	0	0	0
	Elliptical	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Round	0	0	0	0	0	0	0	1	0	1	1	1	1	1
51. Shape of the outer sheath	Oblong	1	0	0	0	0	1	1	0	1	0	0	0	0	0
	Elliptical	0	1	1	1	1	0	0	1	0	1	1	1	1	1
52. Extend of outer sheath	Incomplete ab/ad 1° Vbs, ab/2°Vbs	1	1	1	1	1	1	0	0	0	0	0	0	0	0
	Incomplete- ab 1° Vbs, ab/2°Vbs	0	0	0	0	0	0	1	0	1	0	0	0	0	0
	Incomplete- ab/2°Vbs	0	0	0	0	0	0	0	1	0	1	1	1	1	1
53. Extend of inner sheath	Complete	0	0	1	1	1	0	0	1	1	0	0	0	0	0
	Incomplete-ab 1° Vbs	1	1	0	0	0	1	1	0	0	0	0	0	0	0
	Incomplete-ab 2° Vbs	0	0	0	0	0	0	0	0	0	1	1	1	1	1
54. Strands of sclerenchyma adaxial	1° Vbs	1	1	1	1	1	1	1	0	1	0	0	0	0	0
	Absent	0	0	0	0	0	0	0	1	0	1	1	1	1	1
55. Girders of sclerenchyma adaxial	Trapezoidal	1	0	0	0	0	1	1	0	0	0	0	0	1	0
	Arched	0	1	1	1	1	0	0	0	1	0	1	0	0	0
	Absent	0	0	0	0	0	0	0	1	0	1	1	1	1	1
56. Strands of sclerenchyma abaxial	2° ,1° VBs	1	1	1	1	1	1	1	0	1	0	0	1	0	0
	2° VBs	0	0	0	0	0	0	0	1	0	1	1	1	1	1
57. Girders of sclerenchyma abaxial	T-shape	1	1	0	0	0	0	0	0	0	0	0	0	1	0
	Below vbs	0	0	1	1	1	0	0	1	1	1	1	1	0	1
	Trabezoidal	0	0	0	0	0	1	1	0	0	0	0	1	0	0
	Arched	0	0	0	0	0	0	1	0	0	1	0	0	0	0
58. Types of bulliform	Irregular	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Regular	1	1	1	1	1	1	1	0	1	0	1	1	1	1
	Fan shape	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Table 6. Macro, micromorphological, and anatomical character states of stem, leaf, and nutlet on the first two principal components axes showing the highest factor loading, factor loading values ≥ ± 0.6 are highlighted.

Characters	Character states	Principal components Factor loading	
		PC1	PC2
1. Stem	Herbaceous	0.30233	0.86008
	Suffrutescent	-0.30233	-0.86008
2. Leaf curvature	Curved	-0.37359	-0.00595
	Straight	0.10115	0.30214
	Curved or straight	0.006515	-0.13643
3. Basilar tufts	Present	-0.32074	0.038987
	Absent	0.32074	-0.03899
4. Lower leaf surface	Glabrous	-0.08297	-0.39842
	Scabrous	0.62215	0.025952
	Pubescent	-0.41329	0.39928
5. Upper leaf surface	Hairy	0.32074	-0.03899
	Scabrous	0.16094	0.26823
	Pubescent	-0.32074	0.038987
6. Leaf sheath	Lower wooly	-0.26095	0.44507
	Glabrous	0.26575	-0.53278
	Pubescent	-0.36057	0.27186
7. Node texture	Glabrous	0.32074	-0.03899
	Bearded	-0.32074	0.038987
8. Internode texture	Glabrous	-0.10202	0.35481
	Wooly	0.45471	-0.70736
	Lower wooly	-0.39999	0.47901
9. Panicle	Tightened	-0.37511	-0.76303
	lax	0.37511	0.76303
10. Central awn feathers	Feathery to the base	0.010182	-0.43577
	Feathery with naked tip	0.17913	-0.28077
	Feathery with naked last part and tip	-0.1702	0.6113
11. Lateral awn	Feathery	-0.39098	-0.68406
	Naked	0.39098	0.68406
12. Lower glume nerve number	Three	0.37138	0.24582
	From 1 to 3	-0.37321	-0.10556
	More than 3	-0.07089	-0.21282
13. Lower glume texture	Glabrous	-0.57503	-0.51457
	Hairy	0.57503	0.51457
14. Upper glume apex	Acute	0.32074	-0.03899
	Obtuse	-0.32074	0.038987
15. Upper glume nerve number	Three	0.28375	-0.23322
	From 1 to 3	-0.35862	0.075126
	More than 3	0.10115	0.30214
16. Upper glume texture	Glabrous	0.27213	-0.54147
	Hairy	-0.27213	0.54147
17. Shape of long cell of abaxial leaf	Rectangular to oblong	0.30125	-0.23351
	Oblong	-0.30125	0.23351
18. Short cell of abaxial leaf	Present	-0.07067	0.39792
	Absent	0.070668	-0.39792
19. Silica cell of abaxial leaf	Present	0.063084	-0.18772
	Absent	-0.06308	0.18772
20. Shape of silica cell of abaxial leaf	Rounded to square	-0.44065	0.28126
	Rod -shape	-0.30233	-0.86008
	Rounded	0.59328	0.11547
	Rounded to rectangular	0.058556	0.24356
21. Prickle of abaxial leaf	Present	0.32074	-0.03899
	Absent	-0.32074	0.038987
22. Macro-hairs of abaxial leaf	Present	-0.43085	0.34162
	Absent	0.43085	-0.34162
23. Stomata of abaxial leaf	Present	0.59094	-0.4058
	Absent	-0.59094	0.4058
24. Shape of long cell of adaxial leaf	Undifferentiated	-0.32074	0.038987
	Rectangular to oblong	0.38365	0.50514
	Oblong	0.058556	0.24356
	Rectangular	-0.30233	-0.86008
25. Wall of long cell of adaxial leaf	Undifferentiated	-0.05007	0.46553
	Undulate	0.37963	-0.51224
	Highly undulate	-0.27715	0.22702
	Straight	-0.32074	0.038987
26. Silica cell of adaxial leaf	Present	-0.05007	0.46553
	Absent	0.050067	-0.46553
27. The shape of the silica cell of the adaxial leaf	Rounded	0.80472	0.20237
28. The prickle of the adaxial leaf	Present	-0.18672	0.33069
	Absent	0.18672	-0.33069
29. Macro hairs of adaxial leaf	Present	0.42035	0.26703
	Absent	0.15712	0.32762
30. Stomata of adaxial leaf	Present	0.64167	0.086114

	Absent	-0.64167	-0.08611
31. The shape of long cell of stem	Undifferentiated	-0.18687	-0.04708
	Rectangular to oblong	0.49787	-0.28346
	Oblong	-0.34909	0.34263
	Undifferentiated	-0.26963	0.41871
32. Wall of long cell of stem	Undulate	0.62634	-0.73498
	Highly undulate	-0.2498	0.46274
	Straight	-0.18687	-0.04708
33. Short cell of stem	Present	-0.48145	0.37433
	Absent	0.48145	-0.37433
34. Silica cell of stem	Present	0.30125	-0.23351
	Absent	-0.30125	0.23351
35. Shape of Silica cell of stem	Square	0.92031	-0.13891
	Rounded to rectangular	0.058556	0.24356
	Rounded	-0.76019	-0.04093
36. Prickle of stem	Present	-0.41333	0.56986
	Absent	0.41333	-0.56986
37. Macro-hairs of stem	Present	-0.45471	0.70736
	Absent	0.45471	-0.70736
38. Stomata of stem	Present	0.60784	0.16328
	Absent	-0.60784	-0.16328
39. Nutlet shape	Narrowly deltoid	-0.32074	0.038987
	Lanceolate	0.57503	0.51457
	Oblong	-0.43178	-0.591
40. Shape of epidermal cell of nutlet	Rectangular to oblong	0.30233	0.86008
	Wrinkled	-0.30233	-0.86008
41. Anticlinal wall of Epidermal cell of nutlet	Undulate	-0.26003	0.72111
	Straight	0.76723	-0.15627
	Highly undulate	-0.53797	-0.5991
42. Silica cell of nutlet	Present	0.62634	-0.73498
	Absent	-0.62634	0.73498
43. Stem shape in T.S.	Ovate	-0.32074	0.038987
	Circular	0.43925	0.47007
	Triangular	0.10115	0.30214
	Rectangular	-0.30233	-0.86008
	Broadly rectangular	-0.18687	-0.04708
44. Outer frame of stem T. S	Waved	0.16161	-0.25106
	Straight	-0.16161	0.25106
45. Vascular bundle line number in stem T. S	Two	-0.33851	0.31983
	Three	0.7617	-0.00276
	Scattered	-0.45683	-0.26251
46. Assimilatory tissue	Narrow	-0.45914	-0.70901
	Wide	0.45914	0.70901
47.Shape of leaf blade in T. S	Round	0.023513	-0.49222
	Elliptical	0.11754	0.40162
	U- shape	-0.19717	0.23853
48.Tertiary vbs	Round	0.37511	0.76303
	Absent	-0.37511	-0.76303
49. Secondary vbs shape	Elliptical	0.92324	0.15718
	Round	-0.92324	-0.15718
50. Primary vbs	Absent	-0.92324	-0.15718
	Elliptical	-0.14723	-0.24613
	Round	0.92324	0.15718
51. Shape of outer sheath	Oblong	-0.5497	0.47598
	Elliptical	0.5497	-0.47598
52. Extend of outer sheath	Incomplete ab/ad 1° Vbs, ab/2°Vbs	-0.73258	-0.45326
	Incomplete- ab 1° Vbs, ab/2°Vbs	-0.26963	0.41871
	Incomplete- ab/2°Vbs	0.92324	0.15718
53. Extend of inner sheath	Complete	-0.39573	-0.39431
	Incomplete-ab 1° Vbs	-0.55824	0.38489
	Incomplete-ab 2° Vbs	0.92204	0.031428
54. Strands of sclerenchyma adaxial	1° Vbs	-0.92324	-0.15718
	Absent	0.92324	0.15718
55. Girders of sclerenchyma adaxial	Trapezoidal	-0.20894	0.30812
	Arched	-0.31086	-0.51307
	Absent	0.92324	0.15718
56. Strands of sclerenchyma abaxial	2° ,1° VBs	-0.74119	-0.19176
	2° VBs	0.92324	0.15718
57. Girders of sclerenchyma abaxial	T-shape	-0.08305	0.038796
	Below Vbs	0.31101	-0.33282
	Trabezoidal	-0.0322	0.3155
	Arched	-0.05007	0.46553
58. Types of bulliform	Irregular	0.058556	0.24356
	Regular	-0.11754	-0.40162
	Fan shape	0.10115	0.30214
Percentage per PCO		25.559 %	18.951 %

The vascular bundle line numbers could clarify its taxonomic importance in identifying *S. plumosa* varieties ; however, it is in two lines in *S. plumosa* var. *seminuda* and three in *S. plumosa* var. *brachypoda*. Assimilatory tissue of the stem is of a high taxonomic value, classifying the taxa under investigation into groups. However, two main groups were recognized based on the thickness of the assimilatory tissue. Group 1) characterized by narrow assimilatory tissue comprising *S. ciliata*, *S. scoparia*, *S. hirtigluma*, and *S. obtusa* (Plate II: 1b, 3b, 10b, 12b), group 2) characterized by wide assimilatory tissue including the rest of the studied taxa (Table 3).

Although, there are limited anatomical studies, including the main types of the family Poaceae leaf blade shapes (Metcalfe, 1960; Doğan, 1982; Doğan & Tosunoğlu, 1992). The outline shape of the *Stipagrostis* leaf blade holds high taxonomic value, distinguishing between *S. plumosa* varieties.*;* However, it is elliptical in *S. plumosa* var. *seminuda* and rounded in *S. plumosa* var. *brachypoda*. De Winter 1965 defined three types of vascular bundles as follows: First-order vascular bundle: possessing proto- and metaxylem vessels; lysigenous cavity usually present; second-order vascular bundle: possessing only metaxylem, with two large vessels, neither protoxylem nor lysigenous cavity present, and third order vascular bundle: xylem and phloem not demarcated or at least large metaxylem vessels absent. The presence of primary vascular bundles helped distinguish the two varieties of *S. plumosa*, as they are present in *S. plumosa* var. *brachypoda* and absent in *S. plumosa* var. *seminuda.*

The bundle sheath of *Stipagrostis* consists of two sheaths: inners of small cells with thickened walls and outer or large, circular thin-walled cells of parenchyma, and this is agreed with previous studies (Jelenc 1950, Brown 1958, De Winter 1965 and Brown 1977). According to Schwendener (1890), the sclerenchymatic zone around the vascular bundles of leaves is a characteristic structure of the family Poaceae. Moreover, Metcalfe (1960) noticed the importance of different sclerenchymatic arrangements in grass taxonomy and grouped these sclerenchymatic regions as I-shaped, T-shaped girders and strands. Furthermore, some family taxa have a continuous sclerenchymatic layer through the abaxial surface between two edges of leaf blades (Metcalfe, 1960; Doğan, 1982). According to Metcalfe (1960), there are also sclerenchymatic cells on the margins of the blades.

Girders or strands shape provides useful taxonomic information in grass leaf blades (Dogan 1982). The essential feature of Kranz's anatomy is that the bundle sheaths contain chloroplast and the chlorenchyma surrounding the outer sheath in a radiate form, consisting of tubular cells (Cerros-Tlatilpa and Columbus, 2009). Chlorenchyma cells have less taxonomic and systematic value among the studied taxa, and our results were incongruent with the previously mentioned studies. Occurrence and the structural details of colorless bulliform cells were also used as a taxonomic character (Metcalfe, 1960; Markgraf-Dannenberg, 1980; Tuan et al., 1965; Jane and Chiang, 1991; Vecchia *et al.*, 1998). Bulliform cells help fold the leaves during water stress to reduce transpiration losses. In arid regions, because of less moisture through vacuoles, bulliform cells help the leaves close as the two edges of leaf blades. If adequate water is available, these cells enlarge, and the leaves open (Metcalfe, 1960) grouped the bulliform cells and pointed out their taxonomical importance.

According to Metcalfe (1960), bulliform cells may be found in both adaxial and abaxial surfaces of leaves. The bulliform cells are of considerable taxonomic significance. They could be used to classify the taxa under investigation into three groups: 1) *S. vulnerans* and *S. plumosa* var. *seminuda* characterized by regular bulliform cells (Plate II: 4b, 8b), 2) *S. multinerva* and *S. hirtigluma* characterized by fan shape bulliform cells (Plate II: 10b, 11b), and 3) irregular in the rest of the studied taxa (Table 4).

Taxonomy must primarily rely on morphological characters to circumscribe taxa (Elkordy et al. 2022a, b). Problems in classification arise when taxa display a large amount of variability due to phenotypic plasticity (van den Berg & Groendijk-Wilders 1999). Boissier 1884 and Bolous 2005 tried to provide a natural system to divide the genus *Stipagrostis* into sections. These studies were based on a small number of morphological characteristics. In the present study, many characters were scored, and numerical methods (UPGMA and PCA) were applied to understand better the relationships between taxa.

Our results show congruence between UPGMA clustering and PCA analysis in suggesting two main clusters, C1 and C2, and three main groups. Cluster (C1) includes one species, *S. ciliata,* which was placed alone in one section (Schistachne), segregation based on the following characteristics: bearded nodes, basilar tufts, obtuse glume apex, absence of prickles in abaxial leaf surface, undifferentiated adaxial leaf surface long cell and narrowly deltoid nutlet. Therefore, the authors highly support the classification of Bolous (2005), in which *S. ciliata* should be placed in a separate section.

Cluster (C2) is split into three main groups. Group A) includes three taxa, *S. scoparia*, *S. vulnerans*, and *S. drarii*. The main characters explaining this segregation (characters with high factor loading ≥± 0.6) are scabrous abaxial leaf surface, rounded shape of the adaxial leaf of silica cell, presence or absence of stomata of the adaxial leaf, undulated wall of stem long cells, the shape of the silica cell of the stem, presence or absence of stomata of the stem, straight anticlinal wall of epidermal cell of nutlet, presence or absence of nutlet silica cell, three vascular bundle lines in stem T. S., secondary V.B.s shape, presence or absence of primary V.B.s, primary V.B.s shape, extend of V.B.s outer sheath form, extend of V.B.s inner sheath form, presence or absence of strands of adaxial sclerenchyma, girders of sclerenchyma adaxial shape, presence or absence of strands of abaxial sclerenchyma.

Group (B) includes six taxa: *S. multinerva, S. plumosa* var*. seminuda, S. uniplumis*, *S. obtusa*, *S. paradisea*, and *S. hirtigluma*. This group is divided into two subgroups, b1, and b2: the first subgroup (b1) includes *S. hirtigluma*, *S. paradisea*, *S. obtusa*, and *S. uniplumis*. This group is characterized by the presence of nutlet silica cells, the undulated wall of the stem long cells, glabrous internodes, lower leaf sheath glabrous, central awn feathery to the base, stomata present in the stem and abaxial leaf surface, straight wall of the long cell of the stem, absence of prickles and macro hairs of the stem, straight anticlinal wall of nutlet epidermal cell, presence of silica cell in nutlet. The second subgroup (b2) includes *S. multinerva* and *S. plumosa* var. *seminuda*. It is characterized by the following: wooly internodes and lower leaf sheath, feathery with naked last part and tip central awn, absence of stomata in stem and abaxial leaf surface, highly undulated wall of the long cell of the stem, presence of prickles and macro hairs of the stem, and silica cell is absent in nutlet.

Group (C) includes four taxa: *S. lanata*, *S. plumosa* var. *brachypoda*, *S. raddiana*, and *S. acutiflora*. This group is characterized by herbaceous or suffrutescent stem, wooly internodes, tightened or lax panicle, feathery with naked last part and tip central awn, feathery or naked lateral awn, rectangular shape of long cell of adaxial leaf, rod-shape of silica cell of abaxial leaf, undulate wall of stem long cell, presence or absence of stem macro-hairs, epidermal cell shape of nutlet, undulate anticlinal nutlet cell wall, presence or absence of nutlet silica cell, rectangular stem shape, assimilatory tissue thickness, presence or absence of tertiary vascular bundles, round shape tertiary vascular bundles.

Generally, our findings strongly support Boulos' (2005) sectional classification particularly based on diagnostic features in the general outline of the stem, pattern of distribution and relationship of vascular bundles, and leaf surface features. . Moreover, according to this study, the authors suggest that the two varieties of *S. plumosa* are best treated as two suitable species, and this needs a future phylogenetic study, including the Egyptian taxa.

CONCLUSION

The current study's findings offer valuable data for evaluating the taxonomy of *Stipagrostis* both at infrageneric, specific, and infraspecific levels. The current study revealed the presence of 14 taxa (12 species and two varieties) for the genus *Stipagrostis* in Egypt with a resurrection of two varieties, *S. plumosa* var. *seminuda* and *S. plumosa* var. *brachypoda*. Likewise, we presented the distinctive features between the closely related taxa of *S. plumosa* var. *seminuda* and *S. plumosa* var. *brachypoda* and the suggestion that they should be treated as two separate suitable species. Our results revealed that the data obtained from the macro-, micro-morphological, and anatomical investigations are congruent with the taxonomic circumscriptions described by the previous authors in Poaceae. Combined with stem and leaf shape, outer frame, sclerenchyma and vascular bundles, assimilatory tissue, the bundle sheath, and bulliform cells, these results will assist at the specific and infraspecific level identification. Their significant value, however, may be more excellent in assessing the infrageneric classification. The anatomical variations of leaf and stem were worthy of consideration because they offer a better understanding of the internal structure and prepare an identification taxonomic key for segregation closely related to taxa. It would be precious if further studies using nutlet, pollen grains, and phylogenetic data were used to validate section boundaries, infrageneric, specific, and infraspecific classification of the genus *Stipagrostis* in Egypt.

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