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ARTICLE

Lemma and palea micromorphological study of *Setaria* species (Poaceae) in Iran

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ABSTRACT *Setaria* (Poaceae, Paniceae, Cenchrinae) comprises 136 species worldwide and 3 species in Iran. This weedy grass is distributed in various parts of the world. In this study, the characters of the lemma and palea of 9 populations of *Setaria* were studied micromorphologically using a scanning electron microscope (SEM) to identify diagnostic characters for the delimitation of the studied species. A total of 18 qualitative and quantitative characters of the lemma and palea surfaces were considered. Different shapes of microhairs, epicuticular wax, and the occurrence of cork cells and silica cells were evident in the studied taxa. The results of this study revealed the taxonomic value of floret micromorphological characters in *Setaria* species.
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Introduction

Setaria P. Beauv., belonging to the Poaceae family, is a cosmopolitan genus comprising 136 annual and perennial species worldwide (POWO 2023). These species are mostly distributed in tropical, subtropical, and warm temperate regions of both hemispheres. Foxtail species thrive in diverse environments, ranging from dry, salty, and rocky areas to coastal regions of rivers and streams. The highest abundance of species is found in the Old World and tropical regions of Africa (Rominger 1962; Morrone et al. 2014).

In Iran, three species of *Setaria* have been recognized: *Setaria viridis* (L.) P. Beauv. and *Setaria verticillata* (L.) P. Beauv., which have a natural distribution, and *Setaria italica* (L.) P. Beauv., which is introduced in Iran. Previously, *Setaria glauca* (L.) P. Beauv. was also considered part of this genus, but it is now a synonym of *Cenchrus americanus* (L.) Morrone, a species also introduced in Iran (Bor 1970; POWO 2023).

Setaria is one of the largest and most taxonomically challenging genera of the Paniceae tribe due to the morphological similarities, highly overlapping morphological traits within and between species, and different ploidy levels (Doust and Diao 2017). The genus shows local adaptations, with locally adapted phenotypes resulting from domestication and selective pressure on weeds in human-impacted habitats. Some species exhibit degrees of resistance to salinity, with a relationship between morphology and functional characteristics observed in resistance to drought and salinity (Muthamilarasan and Prasad 2017). The successful occupation of habitats by these species is related to human activities, agriculture, and weed control operations. The phenotypic and genotypic diversity has allowed non-invasion, colonization, and adaptation of these species to a wide range of degraded and damaged habitats (Dekker 2003). Some species of this genus have high economic importance. For instance, *S. italica* is cultivated as a source of seed for its nutritional value in large parts of India, China, South Korea, and Japan, while other species are invasive weeds (Dekker 2003; Pant et al. 2016).

Previous studies have confirmed the taxonomic value of micromorphological characters in Poaceae members. Ortúñez and Cano-Ruiz (2013) studied the micromorphological characters of the lemma, palea, and leaf blade in *Festuca* L. species, helping to separate different sections within the *Festuca* subgenus. Olonova et al. (2016) studied lemma micromorphology in Siberian species of *Stipa* L., showing that sectional boundaries within this genus should be revised. Using lemma micromorphological characters, Wróbel et al. (2017) demonstrated the importance of these features in separating *Eragrostis* Wolf species. Mosaferi and Keshavarzi (2021) studied the lemma and palea surfaces of *Bromus* L. species using SEM,

Table 1. Voucher details of studied populations

Taxon	Locality and herbarium number
Setaria italica	Tehran, West of Tehran, Chitgar, 35 º43'37.04" N., 51º12' 51.44" E., 1278 m., 79/29ALUH
S. verticillata	Sistan & Baluchestan, Barahui, 31º 8'37.01" N., 61º47'28.67" E., 483 m., 77/25 ALUH
	Isfahan, Isfahan, 32.67 º N., 51.67º E., 1575 m., 80/99 ALUH
S. viridis	Alborz, Karaj, 35º51' N., 50º44' E., 1760 m., 6/16 ALUH
	Isfahan, Kashan, 33.98º N., 51.44º E., 944 m., 17/66 ALUH
	Sistan & Baluchestan, Barahui, 30.87º 8'36" N., 61.63º45'26" E., 487 m., 7/26 ALUH
Cenchrus americanus	Markazi, Delijan, 34º0'2.87" N., 50º 40'22.77" E., 1514 m., 69/51 ALUH
	Mazandaran, Sari, 36º 34'26.36" N., 53º5'11.65" E.34.7 m., 61/43 ALUH
	Alborz, Mahdasht, 35º43'7.31" N., 50º 48'4.43" E., 1162 m., 64/46 ALUH

confirming sectional levels within this taxon.

Limited micromorphological studies have been conducted on *Setaria* species. Micromorphological leaf features such as diversity in short and long cells, silica bodies, and micro and long hairs, as well as the presence or absence of stomata and the shape of subsidiaries, were among the diagnostic features used to identify different *Setaria* species (Keshavarzi and Seifali 2005). Lu et al. (2009) investigated the microscopic silica bodies and their diagnostic value in distinguishing *Setaria italica* from *Panicum miliaceum*, highlighting the importance of cruciform silica bodies, the arrangement of papilla hairs, the condition of long cell walls, and the pattern of surface decorations.

Due to the importance of these plants and the need to separate different species, this research discusses the separative value of lemma and palea micromorphological features to improve species delimitation in this genus.

Materials and Methods

Sample collection

Samples were collected from natural habitats throughout Iran (Table 1). Voucher specimens are deposited at the Alzahra University Herbarium (ALUH). In total, nine accessions of three *Setaria* species, along with *C. americanus*,

Table 2. Qualitative and quantitative characters studied

Lemma No.	Character	Character states
1	Length/width of lemma	Mean value in µm
2	Lemma apex	Pointed (1), not so (2)
3	Lemma's nerve	Prominent (1), not so (2)
4	Long cell outline	Straight (1), wide U-shaped (2), Ω-shaped (3)
5	Silica cell	Absent (1), present (2)
6	Shape of silica cell	Dumbbell-shaped (1), saddle-shaped (2)
7	Cork cell	Absent (1), present (2)
8	Papillae	Absent (1), present (2)
9	Bicellular microhair	Absent (1), present (2)
10	Shape of bicellular microhair	Long-base cell (1), short-base cell (2)
11	Wax shape	Wrinkled film (1), film (2), globular (3), cubic (4)
Palea No.	**Character**	**Character states**
1	Length/width of palea	Mean value in µm
2	Palea apex	Pointed (1), not so (2)
3	Long cell outline	Straight (1), wide U-shaped (2)
4	Cork cell	Absent (1), present (2)
5	Papillae	Absent (1), present (2)
6	Bicellular microhair	Absent (1), present (2)
7	Wax shape	Wrinkled film (1), globular (2), cubic (3)

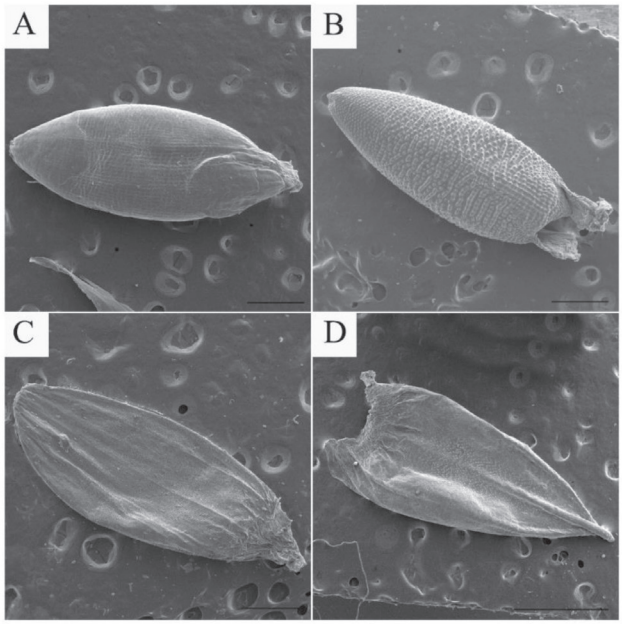

Figure 1. SEM micrographs of the lemma in A: *S. italica* (Delijan population); B: *S. verticillata* (Isfahan population); C: *S. viridis* (Karaj population); D: *Cenchrus americanus*. Scale bar = 500 µm in A, B, C, and 1 mm in D.

were gathered from various locations. The lemma and palea surfaces of mature flowering plants were examined, focusing on the lowermost spikelet.

Sample preparation

To obtain proper micrographs, the samples were prepared as follows: The specimens were transferred directly to aluminum stubs. They were then coated with gold under a high vacuum.

The prepared samples were examined using TESCAN electron microscope (Tescan, Brno).

Micromorphological characters

The evaluated micromorphological characters for lemma and palea are detailed in Table 2. The terminologies of Ellis (1979), Snow (1996), Liu et al. (2010), and Ortúñez and De la Fuente (2010) were used for the description of lemma and palea surfaces. Epicuticular wax terms were described using the guidelines of Consaul and Aiken (1993) and Barthlott et al. (1998).

Statistical analysis

To detect significant differences in the studied characteristics of each species, an analysis of variance (ANOVA) was performed. For revealing species similarity based on palea and lemma micromorphological features, cluster analysis and principal component analysis (PCA) were conducted.

Quantitative and qualitative data analysis

The mean of quantitative characteristics was used for analysis. Variables were standardized for multivariate statistical analyses. Qualitative features were encoded as binary/multi-state features.

Cluster analysis

The average taxonomic distances and squared Euclidean distances were employed as the dissimilarity coefficients in the cluster analysis. A selected set of data was used to prepare the dendrogram, which was based on the micromorphological features of the samples. PAST ver. 4.03 software was utilized for these analyses (Hammer et al., 2001).

Principal Component Analysis (PCA)

PCA was performed to identify patterns and relationships among the studied species. This analysis helped in understanding the variance and grouping based on the micromorphological characteristics of lemma and palea surfaces.

Table 3. Summary of evaluated lemma characters in species studied

Species	Population	Character no.										
		1	2	3	4	5	6	7	8	9	10	11
S. italica	Tehran	2.509	1	2	1	1	-	1	1	2	1	1
S. verticillata	Barahui	2.722	1	2	3	2	1	1	1	2	2	3
	Isfahan	2.670	1	2	1	1	-	1	2	1	-	2
S. viridis	Barahui	2.138	2	2	3	2	1	1	1	1	-	3
	Karaj	2.457	1	2	3	2	1	1	1	2	1	4
	Kashan	2.465	1	2	2	2	1	2	1	1	-	4
C. americanus	Delijan	2.468	1	1	3	2	2	1	2	2	1	3
	Sari	2.467	1	1	3	2	2	1	2	2	1	3
	Mahdasht	2.471	1	1	3	2	2	1	2	2	1	3

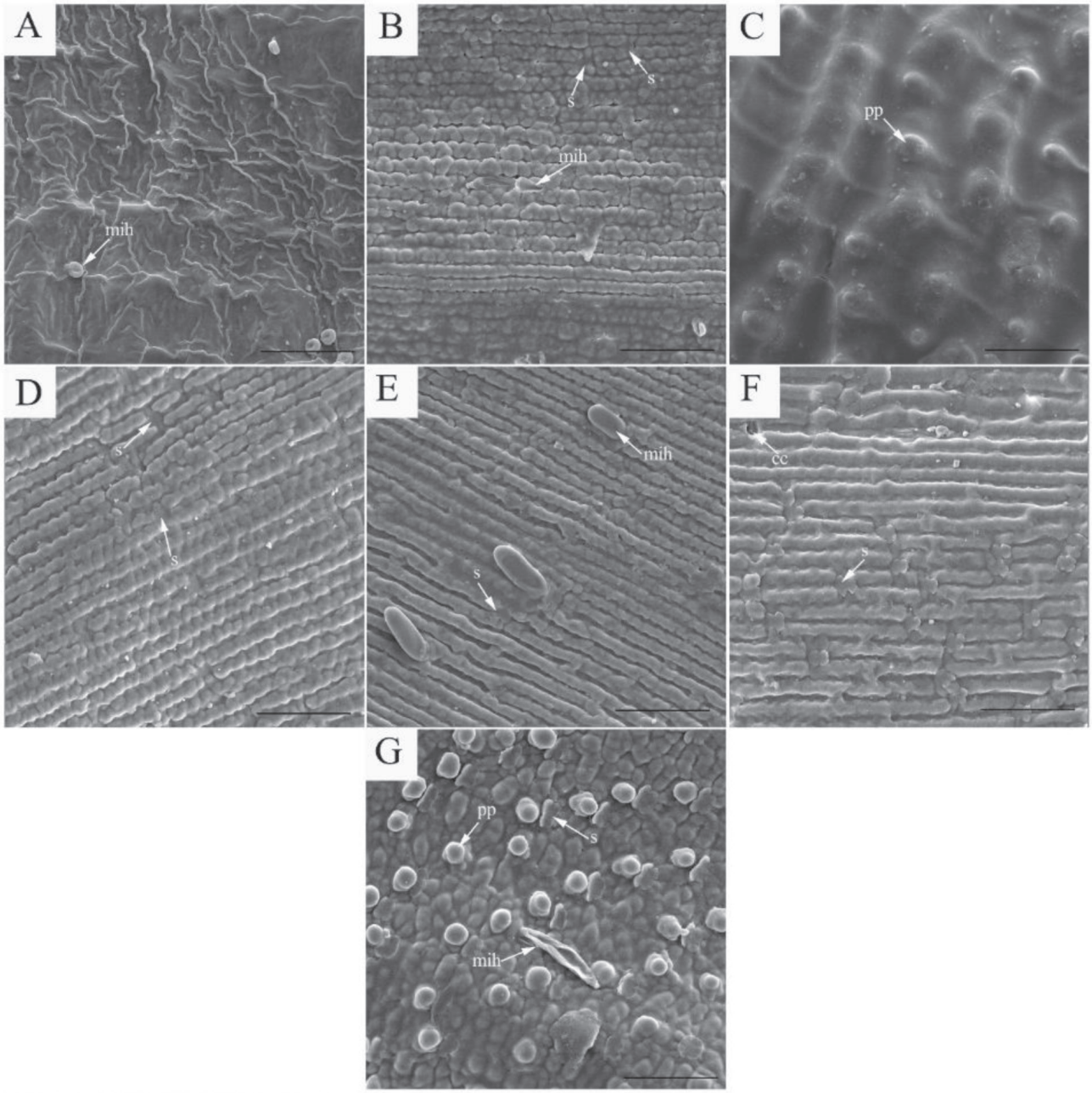

Figure 2. SEM micrographs of lemma surface in A: *S. italica*; B: S. verticillata (Barahui population); C: *S. verticillata* (Isfahan population); D: *S. viridis* (Barahui population); E: *S. viridis* (Karaj population); F: *S. viridis* (Kashan population); G: *Cenchrus americanus*. Scale bar = 50 μm. Abbreviations: cc = cork cell; mih = microhair; pp = papillae; s = silica cell.

Results

Lemma micromorphology

The studied taxa had nearly the same size of lemma. The ratio of length to width of lemma varied between 2.14 (Barahui population of *S. viridis*) and 2.72 μm (Barahui population of *S. verticillata*). Among the studied taxa, lemma nerve was only observed in populations of C. americanus (Fig. 1). All populations had pointed apexes in the lemma except the Barahui population of *S. viridis* (Table 3).

Micrographs of lemma showed Ω-shaped outlines in long cells of most taxa/populations studied, except in *S. italica*, the Isfahan population of *S. verticillata*, and the

Table 4. Summary of evaluated palea characters

Species	Population	Character no.						
		1	2	3	4	5	6	7
S. italica	Tehran	2.475	1	1	1	2	1	2
S. verticillata	Barahui	2.148	1	2	2	2	1	2
	Isfahan	2.137	1	2	1	2	1	2
S. viridis	Barahui	1.925	1	1	1	2	1	2
	Karaj	1.928	1	1	1	1	1	1
	Kashan	1.931	1	1	1	2	1	2
C. americanus	Delijan	1.934	1	1	1	2	2	3
	Sari	1.930	1	1	1	2	2	2
	Mahdasht	1.925	1	1	1	2	2	2

Kashan population of *S. viridis*. Silica cells were found in all taxa except *S. italica*. They were saddle-shaped in *C. americanus* but dumbbell-shaped in other taxa. Cork cells with a semi-circular shape were only observed in the Kashan population of *S. viridis*. A few bicellular microhairs were detected in all taxa. Microhairs with long basal cells were observed in *S. italica*, *S. viridis* (Karaj population), and *C. americanus*. The Barahui population of *S. verticillata* showed bicellular microhairs with short basal cells (Fig. 2). Papillae densely covered the lemma surfaces of *S. verticillata* (Isfahan population) and *C. americanus*, where they were prominent with rounded, swollen tips. Epicuticular waxes of different shapes and sizes were evident in all studied species (Table 3).

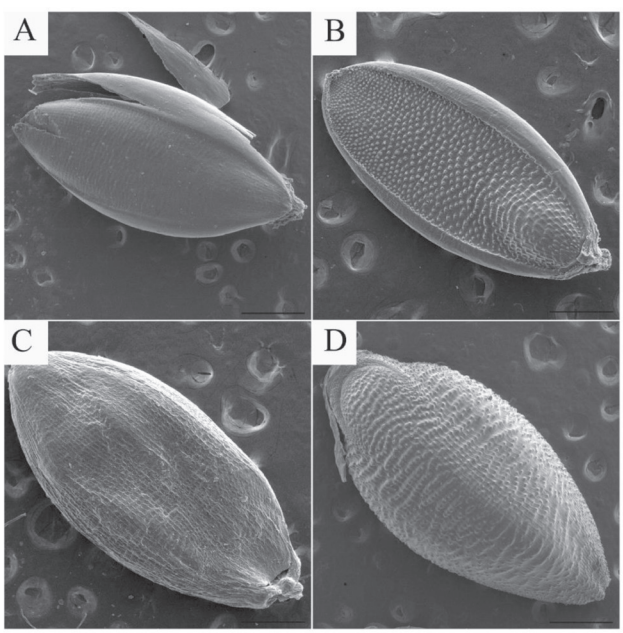

Figure 3. SEM micrographs of the palea in A: *S. italica* (Delijan population); B: *S. verticillata* (Isfahan population); C: *S. viridis* (Karaj population); D: *Cenchrus americanus*.

Palea micromorphology

The highest ratio of length to width of lemma was observed in *S. italica*, while the lowest ratios were observed in *S. viridis* (Barahui population) and *C. americanus* (Mahdasht population) (Table 4). The palea of all studied taxa had pointed apexes (Fig. 3). Except for *S. verticillata*, which had a wide U-shaped outline in long cells, other taxa had long cells with nearly straight outlines. Cork cells were absent in the studied taxa except in the Barahui population of *S. verticillata*. Papillae were observed in all taxa except the Karaj population of *S. viridis*. They varied in size and frequency and were densely scattered on the palea surface of the Isfahan population of *S. verticillata* and two populations of *S. viridis* (Figure 4). Bicellular microhairs with short basal cells were only observed in *C. americanus*.

Statistical analyses

The results of UPGMA clustering based on Gower's distance showed two main clusters (Fig. 5). The first main cluster comprised *C. americanus* accessions, while the second one consisted of *Setaria* species. In the second main cluster, *S. viridis* and the Barahui population of *S. verticillata* formed the first separate subcluster, while *S. italica* was nested near the Isfahan population of *S. verticillata*.

Discussion

Species of Panicoideae are predominantly distributed in tropical to warm temperate habitats (Soreng et al. 2015). *Setaria* is widely distributed due to its vast adaptation to environmental fluctuations, including tolerance to many herbicides, salt, mechanical damage, and drought (Dekker 2003). Their breeding system, predominantly self-pollination, and small genome size result in highly

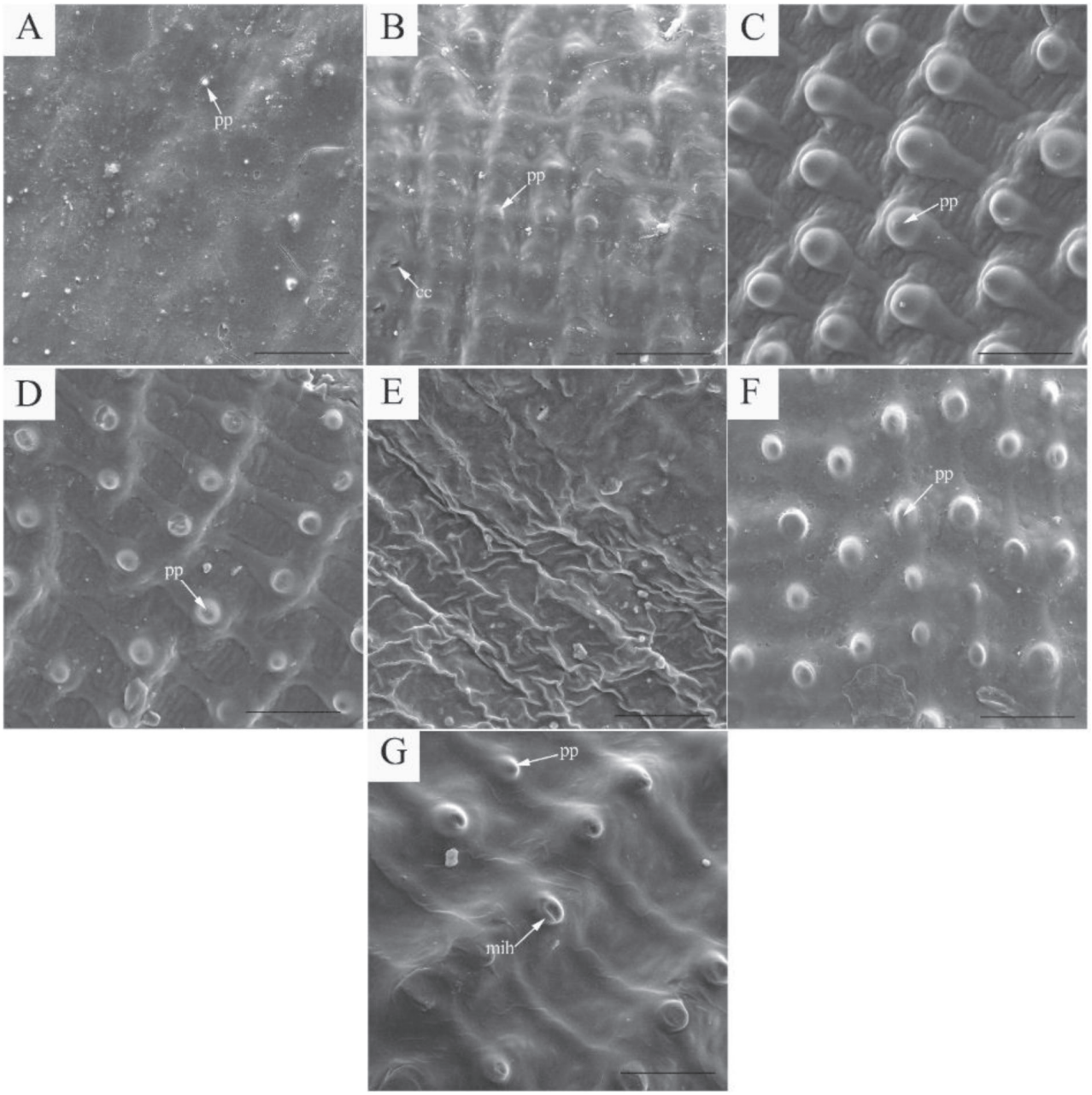

Figure 4. SEM micrographs of palea surface in A: *S. italica*; B: S. verticillata (Barahui population); C: *S. verticillata* (Isfahan population); D: *S. viridis* (Barahui population); E: *S. viridis* (Karaj population); F. *S. viridis* (Kashan population); G: *C. americanus*. Scale bar = 50 µm. Abbreviations: cc = cork cell; mih = microhair; pp = papillae.

diversified, locally adapted genotypes and phenotypes (Dekker 2003).

In the *Setaria* species group, various data sources have proven efficient for species separation. Leaf anatomical features were used by Keshavarzi and Seifali (2005) to differentiate *Setaria* species in Iran. Shaheen et al. (2011) employed palynological and anatomical features within the same genus. Keshavarzi and Seifali (2007) used morphological features as diagnostic tools for *Setaria* species in Iran. High similarities were found between *S. viridis* and *S. verticillata*, while *C. americanus* was clearly separated by its wrinkled upper lemma. Some confusion between *S. verticillata* and *S. viridis* was attributed to hybridization events (Keshavarzi and Seifali 2007). Field observations

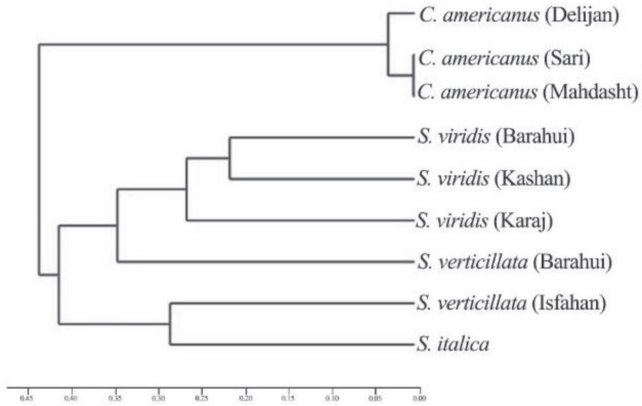

Figure 5. UPGMA dendrogram based on micromorphological characters.

are proposed for a better evaluation of these characters.

The value of micromorphological features in Poaceae is well-supported (Klimko et al. 2015; Aliscioni et al. 2016). Wang and Henwood (1999) used such features for *Elymus* delimitation. Lu et al. (2009) used lemma silica bodies to separate different *Setaria* species. In the present study, *Setaria* species in Iran were examined for variation in their lemma and palea micromorphological features to determine their diagnostic value. Our findings support the close similarity between *S. viridis* and *S. verticillata*, and the distinction of *C. americanus* from other studied species due to the shape of silica cells and the presence of papillae in the lemma and microhairs in the palea.

Layton and Kellogg (2014) considered *S. viridis* and related species, finding that this species is diploid and the progenitor of many polyploids within the genus. Further studies should consider the genetic structure of this widely distributed and somewhat invasive genus to better understand its gene pool for crop improvement.

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