Micromorphological variation forage crops of Loliinae subtribe (Poaceae)

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Original Article

Keywords: Loliinae, micromorphology, silica bodies, lemma, palea, epicuticular wax.

Posted Date: April 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-20606/v1>

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Abstract

Background: Loliinae subtribe (Poaceae) composed of famous temperate grasses with forage and ornamental importance worldwide. Some elements as *Lolium*, *Festuca* and *Vulpia* are widely used as fodder and ornamental in gardens. In present project micromorphological characters of lemmas and paleas using scanning electron microscopy for 56 taxa of 32 species of Loliinae, have been investigated. In present project micromorphological characters of lemmas and paleas using scanning electron microscopy for 56 taxa of 32 species of Loliinae, have been investigated. By evaluating these set of features in abaxial lemma and palea, we aimed to clarify the taxonomic value in this subtribe for the first time. Other purpose of present study was to assess the impact of silica bodies and epicuticular wax in species for livestock palatability.

Results: Features studied including long cells length, long cells shape, periclinal wall of long cells, short cells, silica cells, prickles, prostration of crown cells thin layer of crown cells, macrohairs and wax shape. Two new types of lemma and palea micromorphological traits were introduced here. Multivariate data analysis was obtained using PAST software.

Conclusions: *Lolium temulentum* is a toxic weed and recorded as dangerous for livestock because of having increased amount of wax and silica bodies at the vegetative parts. Taxa relationships were discussed based on micromorphological characters and the results were in concordance with the previous molecular findings. Although some genera studied, were not grouped properly.

Background

Pooideae Benth is the largest subfamily that comprises approximately 202 genera, 15 tribes, 30 subtribes, and 3968 species (Soreng et al. 2017). This subfamily is applied in cattle feeding, as crops of nutritional and medicinal importance for human and ornamental in gardens such as *Festuca ovina*, *Lolium multiflorum*, *L. perenne*, *Vulpia myuros* and *V. bromoides* (Hockenberry Meyer et al. 1998, Loo 2005). The tribe Poeae includes about 135 genera characterized by two to several -flowered spikelets, generally arranged in panicles (Decker 1964).

In this tribe there are many subtribes, one of which is the Loliinae. Tzvelev (1982) subdivided it into 9 genera *Festuca* L., *Lolium* L., *Vulpia* C.C. Gmelin, *Nardurus* (Bluff. Ness and Schauer) Reichenb, *Loliulom* L., *Scleropoa* Griseb., *Cutandia* Willk, *Sphenopus* Trin, and *Bellardiochloa* Chiov.. According to Soreng et al. (2017) with phylogenetic classification divided it into *Castellia* Tineo, *Drymochloa* Holub, *Festuca*, *Leucopoa* Griseb., *Lolium*, *Megalachne* Steud., *Patzkea* G. H. Loos, *Podophorus* Phil. and *Pseudobromus* K. Schum..

Loliinae, as one of the largest subtribes of temperate grasses, is distributed almost worldwide (Minaya et al., 2017). Among the genera of Loliinae, some are more important and widespread as *Lolium* (8 sp.), *Festuca* (500 sp.) and *Vulpia* (25–30 sp.). These annual and perennial grasses are of fodder and weed importance with limited medicinal applications.

Although some of these genera are easily distinguished but some show morphological similarities and overlaps in features. The *Festuca-Lolium* complex can be considered as group of taxa belonging to *Festuca* and *Lolium* with closely related. Even there is hybridization between two latter genera (*Lolium* and *Festuca*) and the x*Festulolium* Aschers. & Graebner. is the result that can be fertile (Clatyon & Renvoize 1986, Jauhar 1993, Wipff 2002). By an inflorescence transformation from panicle to spike, *Lolium* is thought to be derived from *Festuca* (Bulińska-Radomska & Lester 1988). Species of *Vulpia* have been shown to hybridize with species of *Festuca* (Ainscough et al. 1986). Phylogenetic studies of *Vulpia* based on nuclear internal transcribed spacer (ITS) and chloroplast DNA (*trnL-F*, *RFLP*) showed that *Vulpia* and *Festuca* were not monophyletic (Darbyshire & Warwick 1992, Catalan et al. 2007). Torrecilla & Catalan (2002) determined two separate evolutionary lineages as fine-leaved and broad-leaved in *Festuca*. The affinity of *Lolium* to the broad-leaved *Festuca* and that of *Vulpia* to the fine-leaved *Festuca* is confirmed (Aincough et al. 1986).

The use of micromorphological features in systematic studies of the Poaceae showed significant diagnostic results in many studies (Metcalf 1960, Palmer & Tucker 1981, Ellis 1986). Different characteristics of leaf blade epidermis (Palmer & Tucker 1983, Thomasson 1984, Barkworth & Everett 1987, López & Devesa 1991, Dube & Morisset 1996a, 1996b, Gilani et al. 2001), spikelets (Terrell et al. 2001), lemmas (Thomasson 1986, Valde's-Reyna & Hatch 1991, Snow 1996, Acedo & Llamas 2001, Pacheco et al. 2003, Wróbel et al. 2017), and paleas (Acedo & Llamas 2001, Ortúñez & Fuente 2010) have been used by scanning electron microscopy (SEM) (Palmer & Tucker 1983, Palmer & Gerbeth-Jones 1986, Dávila & Clark 1990) for distinguishing taxa within Poaceae, especially in subfamily and tribal levels.

There are many traits that are of taxonomic significance as: shape and distribution of silica bodies (Palmer & Tucker 1981), long and short cells, different hair types as prickles, macrohairs and crown cells especially in the lemma and palea surface (Giraldo- Cañas et al. 2012; Nobis et al. 2016, Ortúñez et al. 2009).

Silica bodies are in fact anti-feedant agent as the grasses with high amount of silica show reduced palatability (Hodkinson 2018). Mainly silica are deposited in vegetative parts but they are also present in reproductive parts as glume, lemma and palea surface. The deposition of silicon caused enhanced strength and rigidity so the water loss via cuticle is decreased. It is especially very functional in tolerance to the lodging, fluctuation in temperature, radiation and drought stresses (Ma and Yamaji 2006).

Silicon is also found in the leaf, glume, lemma and palea margins as siliceous trichomes. Control of temperature along drought periods and higher temperature periods with high evaporation rate is a proposed role for the silica bodies.

Epicuticular wax which was thoroughly reviewed and classified Barthlott et al. (1998), is a functional tool to overcome aridity of the environment by decreasing the water loss via epidermis surface and stomata. They pointed to 23 different types of epicuticular wax. In the Poaceae family, diketone-tubules, platelets, longitudinally aggregated rodlets are mainly observed (Barthlott et al. 1998).

Epidermal micromorphology of lemma and palea has not been widely studied in the Pooideae, especially in the Loliinae. Bodoux (1971) considered *Lolium multiflorum* Lam., *F. pratensis* Hudon, *Festuca arundinacea* Schreber, and their hybrids, and found that two classes, based on epidermal traits in the intercostal regions, could be separated based on the shape of cell wall and the existence of cork cells. Aiken and Lefkovitch (1984) studied the Canadian rough fescue complex (*F. campestris*, *F. hallii*, *Festuca altaica*, *F. scabrella*), and Dube´ & Morisset (1996a, b) studied in micromorphology of *F. rubra* s.l.. Indumentum and shape of the lemma in *Vulpia* have been studied (Brullo et al. 2003).

Some species belong to sections *Schedonorus*, *Eskia* and *Festuca* (Ortúñez & De la Fuente 2010) have been studied and showed the taxonomic value of several characteristics.

The genera of the subtribe Loliinae have not been the subject of any extensive micromorphological examinations; therefore, in present study 56 taxa of 32 species of Loliinae, particularly in terms of the lemma and palea micromorphological features were considered. In present study lemma and palea comparative micromorphological structures in the genera of the subtribe Loliinae, are examined to assess their taxonomic value.

Material And Methods

Plant material

The micromorphology of the lemma and palea epidermis of 56 taxa representing the 32 genera of subtribe Loliinae were obtained from the following herbaria: ALUH (Alzahra University), IRAN (Herbarium Ministerii Iranici Agriculturae, Department of Botany), FUMH (Mashhad University Herbarium), ISTO (Istanbul University Cerrahpasa, Faculty of Forestry, Forest Botany department) and ISTE (Herbarium of the Faculty of Pharmacy of Istanbul University). A list of species and outgroup studied (*Phalaris minor*) is mentioned in Table 1. This table include the authors of scientific names of the samples which lemmas and paleas abaxial surfaces were scrutinized by means of scanning electron microscope (SEM). Adopted scientific names were based on The Plant List (TPL, www.theplantlist.org). Lemmas and paleas were collected from the two first florets of mature spikelets. The middle part of lemma and palea were examined.

Table 1

List of studied species of the subtribe Loliinae used in the micromorphological examination

No.	Taxon	Herbarium number	No.	Taxon	Herbarium number
1	<i>Lolium rigidum</i> Gaudin	ALUH-1054	29	<i>F. pratensis</i>	IRAN-74197
2	<i>L. rigidum</i>	ALUH-1025	30	<i>F. skvortsovii</i> E.B.Alexeev	IRAN-48897
3	<i>L. multiflorum</i> Lam.	ALUH-1046	31	<i>F. sulcata</i> (Hack.) Beck	IRAN-20486
4	<i>L. multiflorum</i>	ALUH-1044	32	<i>F. sulcata</i>	IRAN-68939
5	<i>L. temulentum</i> L.	ALUH-1055	33	<i>F. tuberculosa</i> (Moris) Coss. & Durieu	Germany
6	<i>L. temulentum</i>	ALUH-1049	34	<i>F. valesiaca</i> Schleich. ex Gaudin	IRAN-48894
7	<i>L. persicum</i> Boiss. & Hohen.	ALUH-1006	35	<i>F. valesiaca</i>	IRAN-48895
8	<i>L. persicum</i>	ALUH-1008	36	<i>F. rubra</i> L.	IRAN-74199
9	<i>L. loliaceum</i> (Bory & Chaub.) Hand.-Mazz.	ALUH-1056	37	<i>F. rubra</i>	IRAN-60977
10	<i>L. perenne</i> L.	ALUH-1016	38	<i>Psilurus incurvus</i> (Gouan) Schinz & Thell.	ALUH-1061
11	<i>L. perenne</i>	ALUH-1057	39	<i>P. incurvus</i>	IRAN-21384
12	<i>L. remotum</i> Schrank	ALUH-1058	40	<i>Vulpia unilateralis</i> (L.) Stace	ISTO-14822
13	<i>Loliolom subulatum</i> (Banks & Sol.) Eig	ALUH-1059	41	<i>V. persica</i> (Boiss. & Buhse) Krecz. & Bobrov	IRAN-75041
14	<i>L. subulatum</i>	ALUH-1060	42	<i>V. persica</i>	IRAN-60975
15	<i>Festuca alaica</i> Drobow	IRAN-48898	43	<i>V. myuros</i> (L.) C.C.Gmel.	IRAN-74576
16	<i>F. arundinacea</i> Schreb.	IRAN-74490	44	<i>V. myuros</i>	IRAN-53904
17	<i>F. beckeri</i> (Hack.) Trautv.	ISTO-34107 (Demir Oral 2010)	45	<i>V. ciliata</i> Dumort.	IRAN-
18	<i>F. brunnescens</i> (Tzvelev) Galushko	ISTO-25747	46	<i>V. ciliata</i>	IRAN-21884

No.	Taxon	Herbarium number	No.	Taxon	Herbarium number
19	<i>F. callieri</i> (Hack.) Markgr.	ISTO-34108 (Demir Oral 2010)	47	<i>V. hirtiglumis</i> Boiss. & Hausskn.	IRAN-75040
20	<i>F. drymeja</i> Mert. & W.D.J.Koch	IRAN-68940	48	<i>V. hirtiglumis</i>	IRAN-59167
21	<i>F. drymeja</i>	IRAN-20460	49	<i>V. bromoides</i> (L.) Gray	ISTO-34136 (Demir Oral 2010)
22	<i>F. heterophylla</i> Lam.	IRAN-10342	50	<i>Festololium-loliaceum</i>	IRAN-20489
23	<i>F. heterophylla</i>	IRAN-74193	51	<i>Festololium-loliaceum</i>	IRAN-20487
24	<i>F. karatavica</i> (Bunge) B.Fedtsch.	FUMH-44869	52	<i>Leucopoa pseudosclerophylla</i> (Krivot.) Bor	IRAN-74498
25	<i>F. karatavica</i>	FUMH-10243	53	<i>L. pseudosclerophylla</i>	IRAN-26357
26	<i>F. ovina</i> L.	IRAN-20466	54	<i>L. sclerophylla</i> (Bisch.) Krecz. & Bobrov	IRAN-20620
27	<i>F. ovina</i>	IRAN-68945	55	<i>L. sclerophylla</i>	IRAN-20618
28	<i>F. pratensis</i> Huds.	IRAN-74489	56	<i>Phalaris minor</i> Retz.	ALUH-70M18

Scanning electron microscopy

For scanning electron microscopy (SEM), the samples were transferred directly to aluminum stubs, coated with gold under high vacuum, and examined with VEGA3 TESCAN scanning electron microscopes (SEM) at Alzahra University, Tehran, Iran. Ten micromorphological characters (nine Qualitative and one quantitative) were examined for both lemma and palea for separation of taxa (Table 2). The quantitative character (length of the long cells) was measured too.

Table 2

Lemma and palea micromorphological qualitative characters and character states in subtribe Loliinae

No.	Char.	Char. state
Lemma		
1	Long cell shape (LC)	1 = rectangular to oblong, 2 = rectangular to square, 3 = oblong
2	Periclinal wall of long cell (PWLC)	1 = straight to U-shaped, 2 = straight, 3 = Ω -shaped, 4 = U-shaped, 5 = V-shaped
3	short cell (CC)	1 = absent, 2 = present
4	Silica cell (SC)	1 = reniform, 2 = elliptical to reniform, 3 = absent, 4 = rounded, 5 = elliptical
5	Prickle (P)	1 = absent, 2 = present
6	Prostration of crown cell (PCC)	1 = pointed to conical, 2 = conical, 3 = pointed, 4 = absent
7	Thin layer of crown cell (TLCC)	1 = wrinkled, 2 = smooth, 3 = absent
8	Macrohair (M)	1 = absent, 2 = present
9	Wax shape(W)	1 = granules, 2 = granules & rodlets, 3 = granules & platelets, 4 = platelets, 5 = rodlets, 6 = granules & crusts
Palea		
10	Long cell shape (LC)	1 = rectangular to oblong, 2 = rectangular to square, 3 = oblong
11	Periclinal wall of long cell (PWLC)	1 = Ω -shaped, 2 = V-shaped, 3 = V-shaped to U-shaped, 4 = U-shaped, 5 = V-shaped to Ω -shaped, 6 = straight
12	Short cell (SC)	1 = absent, 2 = present
13	Silica cell (SC)	1 = absent, 2 = reniform, 3 = rounded, 4 = elliptical, 5 = triangular-rounded, 6 = rounded to reniform
14	Prickle (P)	1 = absent, 2 = present
15	Prostration of crown cell (PCC)	1 = pointed, 2 = pointed to conical, 3 = conical, 4 = absent
16	Thin layer of crown cell (TLCC)	1 = wrinkled, 2 = smooth, 3 = absent
17	Macrohair (M)	1 = absent, 2 = present
18	Wax shape (W)	1 = granules, 2 = granules & platelets, 3 = granules & rodlets, 4 = platelets, 5 = rodlets

In general, terminology of Metcalf (1960), Palmer and Tucker (1983), Barthlott (1981), snow (1996), and Barthlott et al. (1998) were followed.

Statistical analysis

In total, two quantitative and 18 qualitative micromorphological features of both lemma and palea surfaces were performed for multivariate analysis containing (Table 2). Multivariate analysis including PCA (Principal component analysis) and UPGMA (unweighted pair group method with arithmetic mean) were performed using PAST ver. 2.17 software, for grouping of the species and obtaining information about general relationships and similarities between them. The data were standardized (mean = 0, variance = 1) for these analyses. Euclidean and taxonomic distance are used among the species (Podani 2000).

Results

Numerical analyses

The numerical analysis of micromorphological qualitative and quantitative features by use of clustering (UPGMA) and ordination (PCA) methods were produced (Figs. 1 and 2). These analyses of the micromorphological characters studied did not support the genera classification and separation of subtribe Loliinae. The genera of subtribe Loliinae were approximately intermixed and they did not delimit properly. Therefore, the genera have high affinity by micromorphological characters. UPGMA tree of micromorphological features revealed that species of the *Lolium* separated from other genera and were placed close to *Festuca*. *Festuca tuberculosa* was placed close to *Festuca sulcata*. The species of the *Vulpia* and *Loliolum* approximately separated and showed high affinity to *Festuca*. *Festololium-loliaceum* taxa were grouped with *Festuca* and *Lolium* species.

Lolium rigidum, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolum subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17) *F. ovina*, 18) *F. pratensis*, 19) *F. skvortsovii*, 20) *F. sulcata*, 21) *F. tuberculosa*, 22) *F. valesiaca*, 23) *F. rubra*, 24) *Psilurus incurvus*, 25) *Vulpia unilateralis*, 26) *V. persica*, 27) *V. myuros*, 28) *V. ciliata*, 29) *V. hirtiglumis*, 30) *V. bromoides*, 31) *Festololium-loliaceum*, 32) *Leucopoa pseudosclerophylla*, 33) *L. sclerophylla*, 34) *Phalaris minor*.

Micromorphological variation of the lemma and palea epidermis

Description of micromorphological features of lemma and palea in subtribe Loliinae

Lemma

The lemma surface was homogeneous and showed epicuticular waxes, with various types and densities. The genera studied illustrated six types of waxes that have been observed in various genera of the subtribe Loliinae (Table 2). *Lolium temulentum* had a high density of wax at the lemma and palea surface.

Long cells varied notably in length. The long cells length (l) varied between 21.31 µm in *Festololium-loliaceum* to 85.99 µm in *Vulpia persica*. The genera studied, showed three types of long cells as rectangular to oblong (Plate1 and 2: 1, 2, 6, 7, 10, 13, 14, 17, 18, 19, 20, 22, 23, 36, 38); rectangular to

square (Plate1 and 2: 3, 4, 5, 35, 37); and oblong (Plate1 and 2: 8, 9, 11, 12, 15, 16, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34). Anticlinal walls also varied in shape and there were five observable types as straight to U-shaped (Plate1: 1, 2, 4, 5); straight (Plate1 and 2: 3, 6, 7, 10, 11, 12, 31); Ω -shaped (Plate1 and 2: 8, 9, 13, 15, 17, 18, 21, 24, 25, 26, 29, 32, 33, 34, 35, 38); U-shaped (Plate1 and 2: 14, 16, 22); and V-shaped (Plate1 and 2: 19, 20, 23, 27, 28, 30, 36, 37).

Two different types of short cells were observed. In first form short cells were filled by suberin (shot cells) and in the second type by silicon (silica bodies).

Silica bodies are valuable character for discriminating and taxonomic purposes. They had different shapes: reniform (Plate1 and 2: 1–6, 8–11, 15, 20, 21, 24, 27, 28, 30, 33–35, 37); elliptical to reniform (Plate1: 7, 13); rounded (Plate1 and 2: 17, 18, 31, 32); and elliptical (Plate 2: 22, 25, 26, 29).

Prickles were observed as cells with swollen bases, hook shaped and thick walls (Plate1 and 2: 8, 9, 15–17, 21, 23, 25, 26, 34, 36, 38). Crown cells are considered as short cells with rounded bases and a conical or pointed prostration that observed in three types as pointed to conical (Plate1 and 2: 1, 6, 8, 10, 12, 14, 15, 21, 22, 25–28, 31–33, 37, 38); conical (Plate1 and 2: 2–4, 7, 30); and pointed (Plate1 and 2: 5, 9, 11, 13, 16–20, 23, 24, 29, 33–35).

Plate1. Micromorphological characters of the abaxial lemma epidermis 1) *Lolium rigidum*, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolom subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17, 18) *F. ovina*, 19, 20) *F. pratensis*.

Plate 2. Micromorphological characters of the abaxial lemma epidermis 21) *Festuca skvortsovii*, 22) *F. sulcata*, 23) *F. tuberculosa*, 24) *F. valesiaca*, 25, 26) *F. rubra*, 27, 28) *Psilurus incurvus*, 29) *Vulpia unilateralis*, 30) *V. persica*, 31) *V. myuros*, 32) *V. ciliata*, 33) *V. hirtiglumis*, 34) *V. bromoides*, 35, 36) *Festololium-loliaceum*, 37) *Leucopoa pseudosclerophylla*, 38) *L. sclerophylla*, 39) *Phalaris minor*.

Palea

The palea surface was homogeneous and showed epicuticular waxes, with different types and densities. The genera studied formed five types of waxes that have been observed in various genera of the subtribe Loliinae (Table 2). *Lolium temulentum* had a high density of wax at the lemma and pales surface. The range of the long cells length in all of the taxa examined was varied between 18.54 μm in *Festuca arundinacea* to 116.38 μm in *Vulpia persica*. The genera studied, showed three types of long cells as rectangular to oblong (Plate 3 and 4: 1, 2, 4, 6, 7, 11, 13, 14, 16–19, 24–28, 31, 36, 38); rectangular to square (Plate 3 and 4: 3, 10, 20, 35, 37); and oblong (Plate 3 and 4: 8, 9, 12, 15, 21–23, 29, 30, 32–34). Five different shapes were observed in anticlinal walls: Ω -shaped (Plate 3 and 4: 1, 2, 6, 7, 9, 10, 15, 17, 18, 21, 22, 27); V-shaped (Plate 3 and 4: 3, 8, 11–13, 19, 20, 28, 29, 34–38); V-shaped to U-shaped (Plate 3: 4, 14); V-shaped to Ω -shaped (Plate 3 and 4: 16, 23–26); and U-shaped (Plate 4: 30–34).

In the present study we observed five different shapes of silica bodies: reniform (Plate 3 and 4: 1–7, 10, 11, 15, 19–21, 37); rounded (Plate 3 and 4: 13, 26); elliptical (Plate 3: 9, 18); triangular-rounded (Plate 3:

17); and rounded to reniform (Plate 4: 37). Results showed that *Lolium temulentum* has a high density of wax and silica bodies at the palea surface.

Prickles were scattered on surfaces in some of taxa studied (Plate 3 and 4: 8, 9, 15–17, 21, 22, 24–26, 28, 33, 35, 38).

Crown cells are common and showed three prostration types as pointed (Plate 3 and 4: 1, 2, 8, 9, 11–13, 15, 17–26, 28–37); pointed to conical (Plate 3 and 4: 3, 6, 10, 14, 16, 27, 38); and conical (Plate 3: 4, 5, 7).

Plate 3. Micromorphological characters of the abaxial palea epidermis 1) *Lolium rigidum*, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolum subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17, 18) *F. ovina*, 19, 20) *F. pratensis*.

Plate 4. Micromorphological characters of the abaxial lemma epidermis 21) *Festuca skvortsovii*, 22) *F. sulcata*, 23) *F. tuberclosa*, 24) *F. valesiaca*, 25, 26) *F. rubra*, 27, 28) *Psilurus incurvus*, 29) *Vulpia unilateralis*, 30) *V. persica*, 31) *V. myuros*, 32) *V. ciliata*, 33) *V. hirtiglumis*, 34) *V. bromoides*, 35, 36) *Festololium-loliaceum*, 37) *Leucopoa pseudosclerophylla*, 38) *L. sclerophylla*, 39) *Phalaris minor*.

Discussion

In the subtribe Loliinae, the lemma and palea micromorphological features have shown to be of taxonomic significance. The lemma and palea epidermal characteristics have demonstrated to be to be taxonomically important and useful. The micromorphological traits among examined taxa, were useful for taxa identification. Results of the UPGMA tree and PCA plot based on the lemma and palea features, revealed that the genera of the subtribe Loliinae were placed close to each other (Figs. 1 and 2).

Results of the UPGMA clustering and PCA plot based on the characteristic of lemma and palea abaxial surface, showed that the clusters of *Lolium* and *Festuca* species were closely arranged due to the shape of silica bodies in palea surface. A controversy in taxonomy relationships between *Lolium* and *Festuca* species have long been remained (Chehrazi et al. 2014).

In these analyses, the clusters of *Festuca*, *Loliolum*, *Psilurus*, *Vulpia* and *Leucopoa* were closely related and their species were not separated efficiently.

Soreng et al. (2017) proposed that three genera as *Loliolum*, *Psilurus* and *Vulpia*, should be considered as synonyms of *Festuca* according to molecular data. Findings of present study are in concordance with Soreng et al. (2017).

Castellia tuberclosa was considered as a separate taxon (Soreng et al. 2017) but our findings support the close relationship of this taxon to the *Festuca* body (*Festuca tuberclosa*).

Lolium species were separated from other genera and placed close to the *Festuca* ones. Same result was achieved by phylogenetic classification using *matK* and *ndhF* plastid DNA markers (Soreng et al. 2017). *xFestulolium* taxa were placed among *Lolium* and *Festuca* species. Also, introgression is possible between these (Wipff 2002).

Lolium temulentum and *L. persicum* (in-breeding species) were placed closed to each other while the cross-breeding species composed separate clusters based on micromorphological characters. *Lolium loliaceum* is considered by Terrell (1968) as synonym of *Lolium rigidum*, but in Flora of Iraq and Flora Iranica (Bor 1968, Bor 1970) is reported as a separate species. Although it is now considered as a synonyme for *L. rigidum* but in accessions studied, *L. loliaceum* and *L. rigidum* were composed separate clusters probably because of different shape of long cells, protrusion of crown cells and thin layer of crown cells in lemma; shape of long cells, anticlinal walls of long cells, short cells, protrusion of crown cells and thin layer of crown cells in palea.

A close relationship was found among the three cross-breeding species (*L. rigidum*, *L. perenne*, and *L. multiflorum*). Although these species are three separate species, however, nowadays it is evident that, these are evolutionary related and show a recent divergence (Abbas et al. 2006).

Species of *Festuca* have been separated to broad-leaved and fine leaved fescues by vegetative and reproductive features (Kar et al. 2019). At the subgeneric level, *Festuca sulcata* and *F. rubra* (Fine-leaved) belonged to the *Festuca* subgenus, grouped as a common main cluster (Fig. 1) while broad-leaved (*F. pratensis*, *F. arundinacea* and *F. drymeia*) ones of *Phaeochloa* subgenus grouped in another main cluster. In section *Festuca*, two species (*F. ovina* and *F. valesiaca*) were located close to each other by features as anticlinal walls of long cells, short cells, protrusions of crown cells, thin layer of crown cells in lemma and anticlinal walls of long cells, short cells and protrusions of crown cells in palea.

Torrecilla and Catalan (2002) determined two separate lineages within two groups of *Festuca* (broad- and fine-leaved). The affinity of *Vulpia* to the fine-leaved *Festuca* was previously stated by Aincough et al. (1986). Soreng considered this genus as a synonym of *Festuca* by molecular findings. Based on present findings, species of *Vulpia* were placed in fine-leaved *Festuca* species cluster.

Results showed that *Lolium temulentum* which is toxic weed, had a high mass of wax and silica bodies at the lemma and palea surface, this makes it dangerous for livestock. Also, some species such as *L. perenne*, *Festuca arundinacea*, *F. ovina*, *F. heterophylla*, *F. skvortsovii*, *F. alaica*, *V. ciliata* and *P. incurvus* had a high density of silica bodies at the reproductive parts. But in other species, a low density of silica bodies observed increased their palatability for livestock. It is believed that in the grass family these are antifeedant agents (Massey & Hartley 2006, 2009).

Silicon-rich species as *Lolium perenne* and *Festuca ovina* were significantly less consumed by locusts than species with low silica content (Massey & Hartley 2009). Hodkinson (2018) reported that silica production in grasses could be an effect of grazing pressure. Herbivores avoid using rough leaves with the high density of silica owing to the harms of feeding. In the Poaceae, reproductive parts (lemma and

palea) showed different shape and density in epicuticular waxes. There are other defensive roles for the epicuticular wax in insect invasion, bacterial influence, ultraviolet radiation and frost (Yang et al. 2015)

Conclusions

In summary, micromorphological features of lemma and palea surfaces were studied using SEM; revealed that the genera of Loliinae subtribe were not separated properly based on these features. At the species level of inbreeder *Lolium* (*L. persicum* and *L. temulentum*) were placed far from cross-breeder ones (*L. perenne*, *L. multiflorum* and *L. rigidum*). Broad-leaved species of *Festuca* have been separated from fine leaved fescues. Also, *Lolium*, *Psilurus*, *Vulpia* and *Leucopoa* were closely related.

The existence of silica and wax in different parts as lemma and palea, was important to adapt to harsh conditions such as endurance to the habitat, alteration in temperature, drought stresses and feeding deterrent to herbivores.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests

Funding

This study was funded by a post-doc agreement with the Alzahra University.

Authors' contributions

Raheleh Tabaripour is a post-doc researcher in Alzahra University working with Maryam Keshavarzi, a faculty member of same university. Both authors collected the samples and studied the variation and contributed in paper writing.

Acknowledgement

We thank curators of the IRAN, FUMH, ISTO and ISTE herbaria for allowing us to use specimens. We also thank the Vice Chancellor for *Research and Technology of Alzahra University* for their supporting.

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Figures

Figure 1

UPGMA tree of the genera of subtribe Loliinae studied based on the micromorphological data. *Lolium rigidum*, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolom subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17) *F. ovina*, 18) *F. pratensis*, 19) *F. skvortsovii*, 20) *F. sulcata*, 21) *F. tuberculosa*, 22) *F. valesiaca*, 23) *F. rubra*, 24) *Psilurus incurvus*, 25) *Vulpia unilateralis*, 26) *V. persica*, 27) *V. myuros*, 28) *V. ciliata*, 29) *V. hirtiglumis*, 30) *V. bromoides*, 31) *Festololium-loliaceum*, 32) *Leucopoa pseudosclerophylla*, 33) *L. sclerophylla*, 34) *Phalaris minor*.

Figure 2

PCA plot of the genera of subtribe Loliinae studied based on the micromorphological data.

Figure 3

Plate1. Micromorphological characters of the abaxial lemma epidermis 1) *Lolium rigidum*, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolum subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17, 18) *F. ovina*, 19, 20) *F. pratensis*.

Figure 4

Plate 2. Micromorphological characters of the abaxial lemma epidermis 21) *Festuca skvortsovii*, 22) *F. sulcata*, 23) *F. tuberculosa*, 24) *F. valesiaca*, 25, 26) *F. rubra*, 27, 28) *Psilurus incurvus*, 29) *Vulpia unilateralis*, 30) *V. persica*, 31) *V. myuros*, 32) *V. ciliata*, 33) *V. hirtiglumis*, 34) *V. bromoides*, 35, 36) *Festololium-loliaceum*, 37) *Leucopoa pseudosclerophylla*, 38) *L. sclerophylla*, 39) *Phalaris minor*.

Figure 5

Plate 3. Micromorphological characters of the abaxial palea epidermis 1) *Lolium rigidum*, 2) *L. multiflorum*, 3) *L. temulentum*, 4) *L. persicum*, 5) *L. loliaceum*, 6) *L. perenne*, 7) *L. remotum*, 8) *Loliolom subulatum*, 9) *Festuca alaica*, 10) *F. arundinacea*, 11) *F. beckeri*, 12) *F. brunnescens*, 13) *F. callieri*, 14) *F. drymeja*, 15) *F. heterophylla*, 16) *F. karatavica*, 17, 18) *F. ovina*, 19, 20) *F. pratensis*.

Figure 6

Plate 4. Micromorphological characters of the abaxial lemma epidermis 21) *Festuca skvortsovii*, 22) *F. sulcata*, 23) *F. tuberculosa*, 24) *F. valesiaca*, 25, 26) *F. rubra*, 27, 28) *Psilurus incurvus*, 29) *Vulpia unilateralis*, 30) *V. persica*, 31) *V. myuros*, 32) *V. ciliata*, 33) *V. hirtiglumis*, 34) *V. bromoides*, 35, 36) *Festololium-loliaceum*, 37) *Leucopoa pseudosclerophylla*, 38) *L. sclerophylla*, 39) *Phalaris minor*.