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ARTICLE

# Anatomical and micromorphological study of *Phalaris* (Poaceae) species in Iran

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**ABSTRACT** The genus *Phalaris*, comprising 18 species globally, has a complex taxonomic history. Species of *Phalaris* hold significant importance as forage and weeds. Anatomical and micromorphological studies can provide diagnostic traits critical for taxonomy. In this study, we investigated the leaf sheath anatomy and the micromorphology of the lemma and palea to identify distinguishing traits among some *Phalaris* species in Iran. Twenty-one accessions of three *Phalaris* species (*P. minor*, *P. brachystachys*, and *P. paradoxa*) from different regions of Iran were studied. Leaf sheath cross-sections were stained using methyl green and Congo red, and both quantitative and qualitative anatomical traits were measured and evaluated. Micromorphological features of the lemma and palea were observed using a Hitachi SU3500 Scanning Electron Microscope. Eleven traits were assessed in the studied species. While the general leaf sheath anatomy showed significant similarities, micromorphological investigations of the lemma and palea epidermis highlighted the palea epidermis, the presence or absence of prickles, and papillae density as key distinguishing features. The findings are consistent with previous studies on this subfamily, enhancing the understanding of *Phalaris* species taxonomy.
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## Introduction

The Poeae tribe, the largest in the subfamily Pooideae, is a key group within the Poaceae family. Historically, the tribe was divided into two groups (Poeae and Aveneae) based on morphological traits. However, phylogenetic analyses, particularly those involving the chloroplast genome, have led to the merging of these tribes. Today, the Poeae tribe is divided into 25 subtribes, encompassing numerous genera with complex taxonomic histories and statuses that have undergone significant revisions (Soreng et al. 2017).

Plants within the Poeae tribe occupy diverse habitats, including deserts, wetlands, agricultural lands, and salt marshes. The genus *Phalaris* (*Phalaridinae*, Poeae, Poaceae) is native to the Mediterranean region and has been traditionally classified based on inflorescence morphology, particularly glumes. Variations in fertile and sterile florets have further refined its taxonomy (Baldini 1993). The genus, with a complex nomenclatural history, includes 18 widely distributed species across temperate and subtropical regions (POWO 2024). Its centers of diversity are in the Mediterranean Basin and western North America.

Phalaris is an excellent genus for studying species diversity, dispersal, hybridization history, polyploidy, and chromosome evolution (Lavergne and Molofsky 2004). The genus is also significant for forage and as a food source for birds (Anderson 1961). In recent decades, some *Phalaris* species have become invasive weeds, posing challenges to wheat cultivation (Kettenring et al. 2019). In Iran, *Phalaris* species are notable weeds in provinces such as Khuzestan, Fars, Golestan, and Mazandaran (Gherekhloo 2008).

Studies on grass anatomy, particularly leaf structure, have proven instrumental in Poaceae taxonomy. For example, Đenader et al. (2020) distinguished three species in the *Sesleria juncifolia* complex based on anatomical traits such as hair length on the leaf ventral surface and sclerenchyma on the dorsal surface. Environmental factors also influence anatomical traits, as demonstrated in *Andropogon gerardii*, where plants in humid areas exhibit larger veins, less hair, thicker mesophyll, and larger bulliform cells compared to those in drier areas (Olsen et al. 2013).

Anatomical features such as the shape of the main vein, vascular bundle number, epidermal cell size, papillae condition, sclerenchyma girders, and bulliform cell presence are critical diagnostic traits (Olsen et al. 2013; Aliscioni et al. 2016; Mavi et al. 2011; Martínez-Sagarra et al. 2017).

Micromorphology also plays a crucial role in distin-

**Table 1.** Voucher details of *Phalaris* taxa used in this study

| Taxon | Locality | Longitude, latitude / altitude | Collector/Voucher no. |
|---|---|---|---|
| *P. paradoxa* var. *praemorsa* | Gilan, Rasht to Tehran Road | 37°03′34.83″ N 49°38′16.38″ E / 104 m | Khaksar/1212 ALUH |
| | Khuzestan, Ramhormoz | 31°17′37.96″ N 49°35′03.87″ E / 141 m | Keshavarzi/85mk43ALUH |
| | Mazandaran, Savadkuh, Zirab | 36°10′29.80″ N 52°58′23.44″ E / 444 m | Khaksar/85pps1ALUH |
| | Mazandaran, Neka, Baye kola | 36°43′59.28″ N 53°14′21.58″ E / -3 m | Khaksar/85pps1 ALUH |
| | Sistan & Baluchestan, Barahui | 31°08′45.31″ N 61°47′44.24″ E / 483 m | Keshavarzi and Ijbari/190mkh ALUH |
| *P. paradoxa* var. *paradoxa* | Khuzestan,15 km to Izeh | 31°46′31.00″ N 49°50′29.70″ E / 827 m | Nanaii, 85pp3ALUH |
| | Mazandaran, Qaem shahr | 36°27′06.30″ N 52°53′02.10″ E / 52 m | Khaksar, 83nkh ALUH |
| | Khuzestan, Ramhormoz, Bayman | 31°16′21.64″ N 49°35′43.14″ E / 151 m | Farasat, 85pp2ALUH |
| | Mazandaran, Qaem shahr to Sari | 36°29′31.70″ N 52°54′50.00″ E / 26 m | Nataj, 853 ALUH |
| *P. brachystachys* | Khuzestan, Izeh, Eshkefte Soleiman | 31°49′01.63″ N 49°50′53.23″ E / 906 m | Keshavarzi, 1383b7ALUH |
| | Sistan & Balushestan, Pasand Khan | 31°06′14.9″ N 61°45′15.90″ E / 485 m | Keshavarzi & Ijbari, 192mkh ALUH |
| *P. minor* | Fars, Shiraz | 29°37′31.08″ N 52°32′24.01″ E / 1500 m | Keshavarzi, 85m10ALUH |
| | Mazandaran, Babol, madar | 36°32′58.88″ N 52°39′39.22″ E / -5 m | Khaksar, 84nkh ALUH |
| | Lurestan, Khorramabad, near Keeyow Lake | 33°30′32.59″ N 48°21′10.35″ E / 1208 m | Keshavarzi, 83m7 ALUH |
| | Gilan, Rasht to Tehran, Saravan | 37°05′39.33″ N 49°39′14.62″ E / 35 m | Nataj, 864nkh ALUH |
| | Yazd, Yazd | 31°54′58.69″ N 54°22′53.98″ E / 1209 m | Keshavarzi, 88Y ALUH |
| | Khuzestan, Izeh | 31°48′56.58″ N 49°51′45.84″ E / 862 m | Keshavarzi, 87A ALUH |
| | Mazandaran, Mahmood Abad, 7 km to Noor | 36°35′22.38″ N 52°05′07.62″ E / -19 m | Nataj, 851ALUH |
| | Mazandaran, East of Behshahr | 36°41′15.76″ N 53°35′23.98″ E / 60 m | Khaksar, 857nkh ALUH |
| | Fars, Sarvestan, Sasanian Palace | 29°11′43.74″ N 53°13′52.71″ E / 1495 m | Keshavarzi, 87SH ALUH |
| | Golestan, Gorgan | 36°51′50.23″ N 54°26′09.15″ E / 74 m | Khaksar, 85m23ALUH |

guishing Poaceae species. Features such as the shape and arrangement of long and short epidermal cells, the type and distribution of hairs, silica body shape, and stomatal number and arrangement are essential diagnostic traits (Mejia-Saules and Bisby 2003; Liu et al. 2010; Shaheen et al. 2011; Ortúñez and De la Fuente 2010; Meng and Mao 2013; Harms and Mendenhall 2015). These traits are used to analyze leaf surfaces and reproductive structures like the lemma and palea, often corroborating molecular findings (Tkach et al. 2021; Tabaripour et al. 2022).

Given the significance of *Phalaris* species as forage, food, and invasive weeds, and considering the morphological similarities among certain species, this study examines the anatomical variation in leaf sheaths and the micromorphology of the lemma and palea. The aim is to identify diagnostic traits and refine species boundaries.

**Table 2.** Qualitative and quantitative anatomical traits of the sheath in *Phalaris* species studied

| Qualitative feature | |
|---|---|
| **Character** | **State of character** |
| Prickle at the dorsal surface | Presence/ absence |
| Prickles at the ventral surface | Presence/ absence |
| Bulliform cells size | Small/ medium/ large |
| Bulliform at the ventral surface | Presence/ absence |
| Bulliform at the dorsal surface | Presence/ absence |
| **Quantitative feature** | |
| Size of central vascular bundle | Width of bulliform cells |
| Length of bulliform cells | Length of prickles |

## Materials and methods

### *Plant material*

In this study, 21 accessions of three *Phalaris* species (*P. minor*, *P. brachystachys*, and *P. paradoxa*) collected from various regions of Iran were analyzed to investigate the anatomical structure of the leaf sheath and the micromorphology of reproductive parts (Table 1). The collected specimens were deposited in the Herbarium of Alzahra University (ALUH).

### *Anatomical study*

To examine the anatomical structure of the leaf sheath, samples were fixed in a water-alcohol-glycerin solution for one week. Hand-cut cross-sections of the sheath were stained with methyl green and Congo red. To remove cell storage materials and tissue coloration, the sections were treated with sodium hypochlorite for 20 min. Prepared samples were observed and photographed using a light microscope at various magnifications. Both quantitative

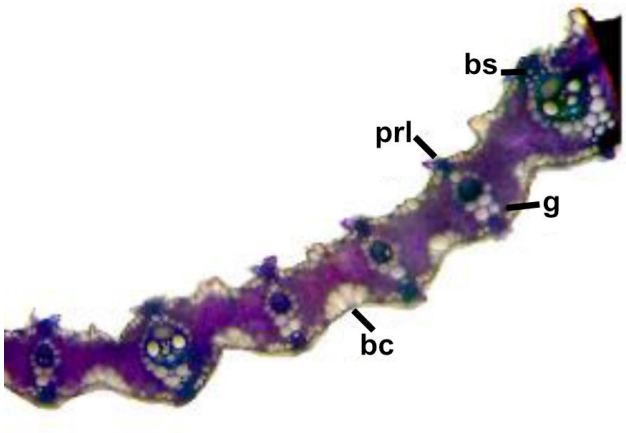


**Figure 1.** Details of the anatomical structure of the sheath in the species studied (bc = bulliform cells; g = girder; pri = prickle; bs = bundle sheath)

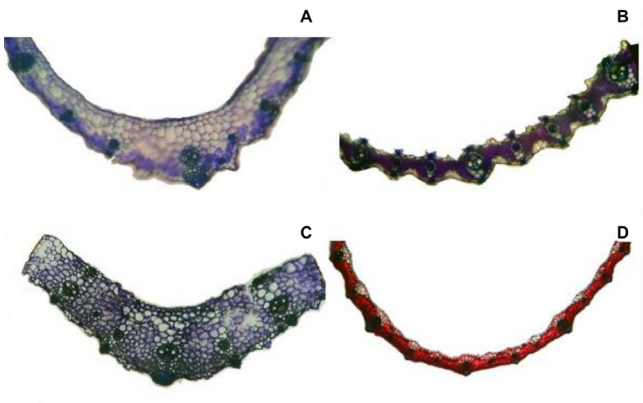


**Figure 2.** Anatomical structure of the sheath. A) *P. brachystachys* (Pas-and Khan population); B) *P. paradoxa* var. *paradoxa* (Qaem Shahr population); C) *P. minor* (Tehran population); D) *P. paradoxa* var *praemorsa* (Rasht to Tehran population).

and qualitative characteristics of the leaf sheath anatomy were measured and analyzed (Table 2).

### *Micromorphological study*

For micromorphological analysis, the samples were placed in boiling water to facilitate the separation of the lemma and palea without causing damage. The separated parts were examined using a stereomicroscope and a Dino-Lite digital stereoscope. The samples were mounted on aluminum stubs, coated with a 100Å gold layer using a sputter coater, and micrographs were taken with a Hitachi SU3500 Scanning Electron Microscope (SEM). A total of 11 quantitative and qualitative traits were assessed in the examined species (Table 3).

## Results

### *Sheath anatomical observations*

The cross-sections of the leaf sheath in the studied species revealed protrusions and depressions on the dorsal surface, while the ventral surface was smooth. Bulliform cells, colorless and varying in length and width, were observed on the dorsal surface. Sclerenchyma tissue surrounded the vascular bundles, differing in size, and a two-layered vascular bundle sheath was identified (Fig. 1).

### *P. brachystachys*

The dorsal surface of this species exhibited ridges, whereas the ventral surface was entirely flat. Bulliform cells were visible on the dorsal surface, and sclerenchyma was concentrated above the vascular bundles and the dorsal surface. Vascular bundles were categorized into three sizes: small, medium, and large (Fig. 2).

The sheath anatomical study revealed that the central vascular bundles were largest in *P. minor* and smallest in

**Table 3.** Evaluated micromorphological traits and their observation results (- = absence; + = presence; +,- = both states).

| Character | Taxon | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *P. minor* | | *P. brachystachys* | | *P. paradoxa* var. *paradoxa* | | *P. paradoxa* var. *praemorsa* | |
| | Palea | Lemma | Palea | Lemma | Palea | Lemma | Palea | Lemma |
| Micropapillae | - | - | - | - | - | - | - | - |
| Hooked papilla | - | - | - | - | - | - | - | - |
| Prickle | + | +,- | + | +,- | +,- | + | + | + |
| Macro-hair | - | - | - | +,- | - | - | - | - |
| Wax | +,- | + | +,- | + | +,- | + | + | + |
| Cork cells | - | - | - | - | - | - | - | - |
| Silica cell | - | +,- | - | + | +,- | +,- | + | +,- |
| Papillae | + | + | + | + | + | + | + | + |
| Micro prickle | - | - | - | - | - | - | - | - |
| Long cell | + | + | + | +,- | + | +,- | +,- | +,- |

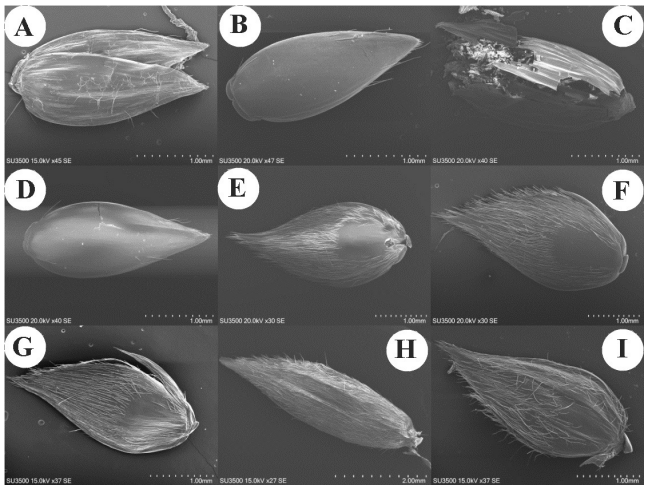


**Figure 3.** Electron micrographs of the lemma in *Phalaris* species. A) *P. paradoxa* var. *praemorsa* (Rasht to Tehran Road population); B) *P. paradoxa* var. *praemorsa* (Ramhormoz population); C) *P. paradoxa* var. *paradoxa* (Qaem Shahr population); D) P. *paradoxa* var. *paradoxa* (Izeh population); E) *P. minor* (Shiraz population); F) *P. minor* (Babol population); G) *P. minor* (Khorramabad population); H) *P. brachystachys* (Izeh population); I) *P. brachystachys* (Pasand Khan population).

*P. paradoxa*. The length and width of bulliform cells were greater in *P. minor* compared to the shorter cells in *P. brachystachys*. Both varieties of *P. paradoxa* exhibited similar bulliform cell dimensions. The vascular sheath consisted of two layers, with smaller, more compact cells in the inner layer. Sclerenchyma tissue was prominently visible at the section margins, and epidermal cells displayed either regular or irregular shapes with equal or unequal sizes. Bulliform cells were hollow, colorless, and varied in number between the dorsal and ventral surfaces (Fig. 2).

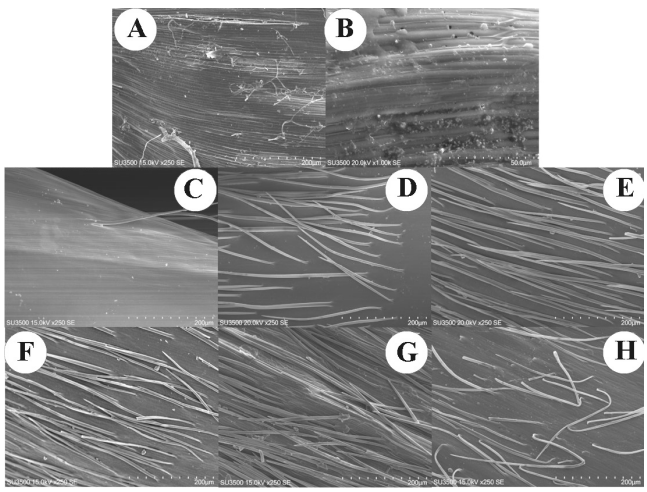


**Figure 4.** Electron micrographs of the lemma surface in *Phalaris* species. A) *P. paradoxa* var. *praemorsa* (Rasht to Tehran Road population); B) *P. paradoxa* var. *praemorsa* (Ramhormoz population); C) *P. paradoxa* var. *paradoxa* (Izeh population); D) *P. minor* (Shiraz population); E) *P. minor* (Babol population); F) *P. minor* (Khorramabad population); G) *P. brachystachys* (Izeh population); H) *P. brachystachys* (Pasand Khan population).

### *P. paradoxa* var. *praemorsa*

The dorsal surface showed ridges across all populations, while the ventral surface was mostly flat, except in the Brahui population (Sistan and Baluchistan). Prickle hairs were present only on the dorsal surface of the Rasht-Tehran population, while other populations lacked prickles. Bulliform cells were grouped in threes and located on the dorsal surface of the sheath. The Brahui population lacked both prickles and bulliform cells (Fig. 2).

### *P. paradoxa* var. *paradoxa*

This variety exhibited vascular bundles with regular shapes, and both surfaces had ridges. The ventral surface was flat in the Izeh and Ramhormoz populations. Sclerenchyma tissue in the Qaem Shahr population was concentrated above the vascular bundles. Pointed prickles were observed only on the dorsal sheath surface in the Ramhormoz population (Fig. 2).

Bulliform cells were present on the dorsal surface in the Ramhormoz population, and on both dorsal and ventral surfaces in the Qaem Shahr population. The Qaem Shahr population exhibited the largest bulliform cells, while the Ramhormoz population had the smallest in both length and width (Fig. 2).

### *P. minor*

In *P. minor*, the dorsal surface of the sheath showed protrusions and depressions. Dense sclerenchyma tissue surrounded all vascular bundles, especially on the dorsal surface. The outer layer of the vascular sheath consisted of large cells, though these were less distinct in some populations. Prickles were absent on both surfaces of the sheath. Bulliform cells were observed on both the dorsal and ventral epidermis, often grouped in threes, fives, or sevens. Bulliform cell sizes ranged from medium (e.g., Gorgan, Tehran to Rasht, Izeh, and Noor accessions) to large (e.g., Yazd and Shiraz populations) to small (e.g., Behshahr population) (Fig. 2).

### *Micromorphological observations*

Surface micromorphology provided key diagnostic features. Micro-papillae, hooked papillae, prickles, and macro-hairs varied in number and size across the lemma and palea (Figs. 3-6). Epicuticular wax showed interspecific differences in type and distribution on the lemma and palea surfaces. The shape and frequency of cork cells, silica cells, and long cells also proved diagnostically significant.

On the lemma surface, the longest long cells were

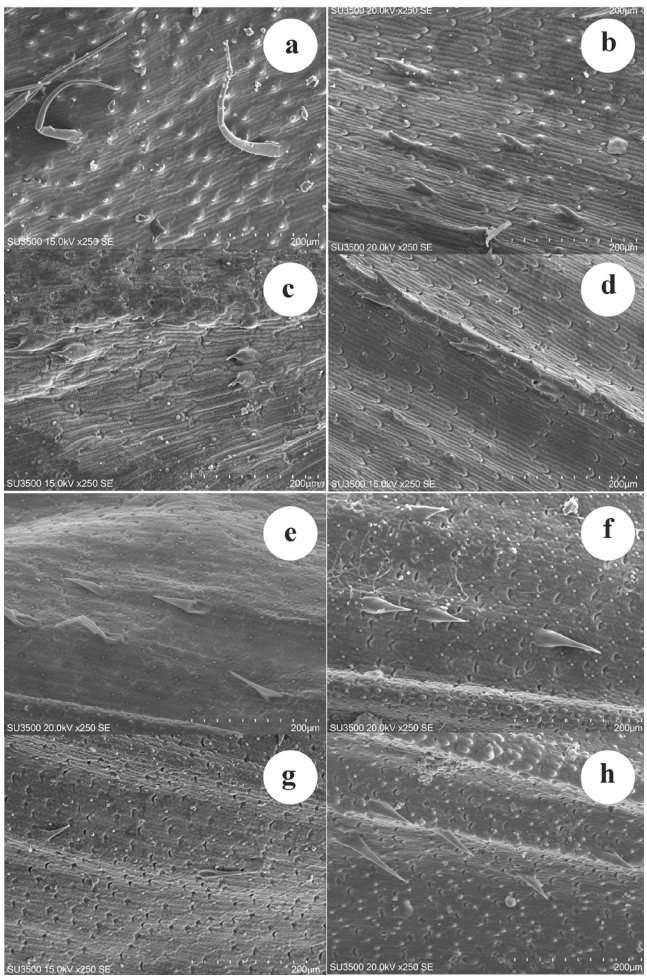


**Figure 5.** Electron micrographs of the lemma surface in *Phalaris* species. a) *P. brachystachys* (Izeh population); c) *P. brachystachys* (Pasand Khan population); b) *P. minor* (Babol population); d) *P. minor* (Khorramabad population); e) *P. paradoxa* var. *paradoxa* (Qaem Shahr population); g) *P. paradoxa* var. *paradoxa* (Izeh population); f) *P. paradoxa* var. *praemorsa* (Rasht to Tehran Road population); h) *P. paradoxa* var. *praemorsa* (Ramhormoz population).

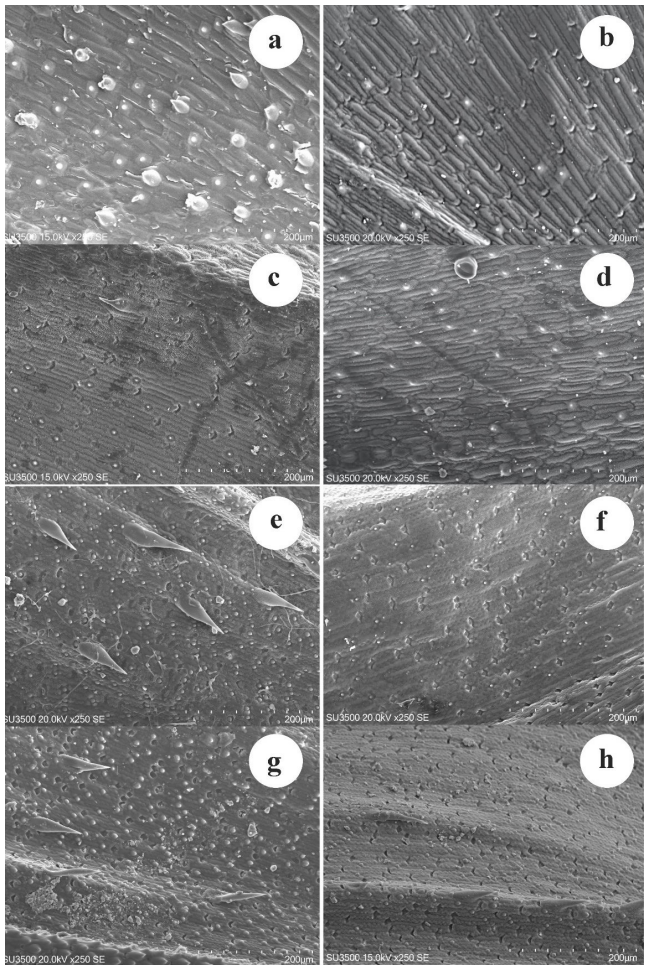


**Figure 6.** Electron micrographs of the palea surface in *Phalaris* species. a) *P. brachystachys* (Izeh population); c) *P. brachystachys* (Pasand Khan population); b) *P. minor* (Shiraz population); d) *P. minor* (Babol population); e) *P. paradoxa* var. *praemorsa* (Rasht to Tehran Road population); g) *P. paradoxa* var. *praemorsa* (Ramhormoz population); f) *P. paradoxa* var. *paradoxa* (Qaem Shahr population); h) *P. paradoxa* var. *paradoxa* (Izeh population).

found in *P. brachystachys* (Pasand Khan population) and the shortest in *P. paradoxa*. The number of prickles in *P. paradoxa* (Izeh population) was lower than in other taxa, while the longest prickles were observed in *P. paradoxa* (Qaem Shahr to Sari population) and the shortest in *P. minor* (Khorramabad population).

Populations of *P. minor* showed papillae and epicuticular wax, with some populations (e.g., Babol and Shiraz) also exhibiting prickles (Table 3). Silica bodies in the Shiraz population were cruciform. Differences in silica body shapes and long-cell sizes distinguished the two varieties of *P. paradoxa*. In *P. brachystachys* (Pasand Khan population), prickles were evident, while macro-hairs appeared in the Izeh population. Epicuticular wax was present on the palea of all studied species, though its ornamentation and size varied.

In the palea, the longest long cells were observed in the Izeh population of *P. brachystachys*, and the shortest in the Ramhormoz population of *P. praemorsa*. The shortest prickles were found in the Izeh population of *P. brachystachys*, while the longest were seen in the Rasht-Tehran population of *P. praemorsa* (Table 3).

Quantitative and qualitative analysis of palea traits revealed that the highest number of prickles occurred in the Saravan population of *P. minor*, while the lowest was observed in *P. brachystachys* (Pasand Khan population). The longest prickles were recorded in the Saravan population, and the shortest in the Khorramabad population.

## Discussion

Extensive macro- and micromorphological studies have been conducted on the Poaceae family, with a focus primarily on reproductive structures such as the lemma, palea, and glume (Acedo and Llamas 2001; Ortúñez and Cano-Ruiz 2013; Harms and Mendenhall 2015; Keshavarzi et al. 2011). These studies highlight the diagnostic value of these traits in distinguishing taxa at various taxonomic levels.

Micromorphological examination of the lemma and palea epidermis in *Phalaris* revealed significant variations among the studied species. Notably, micro-prickles and hooks were absent from the lemma and palea surfaces of all species examined. The species exhibited diversity in the length of long cells, although populations of *P. minor* showed minimal differences in qualitative traits. Previous studies on different populations of *P. minor* in Iran reported notable variations in morphological and anatomical characteristics, suggesting the presence of distinct ecotypes (Keshavarzi et al. 2007). However, the findings of the current study do not align with these earlier observations.

The shape of prickles on the lemma surface varied among the species. In the Rasht to Tehran Road population of *P. praemorsa*, prickles were broad at the base with a curved tip. In the Pasand Khan population of *P. brachystachys*, prickles were sharp and pointed, whereas in *P. minor*, they had a distinctly curved tip. Similar epidermal variations in prickle shape and size across members of the Pooideae have been noted by Desai and Raole (2013).

Among the distinguishing micromorphological traits, the palea epidermis, the presence or absence of prickles, and the density of papillae emerged as the most critical characteristics for separating the studied species. The highest papilla density was observed in the Ramhormoz population of *P. praemorsa*, while the Izeh population of *P. paradoxa* exhibited the lowest density.

Overall, the findings of this study underscore the significance of micromorphological traits in distinguishing *Phalaris* species. These results are consistent with previous research within the Pooideae subfamily, reinforcing the taxonomic importance of such traits in refining species boundaries.

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