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Systematic Study of Annual Weed *Phalaris minor* Retz. (Poaceae) in Iran

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Abstract: *Phalaris minor* is an annual grass in North temperate regions. This weed is inhabited in damp soils and swamps of Irano-Turanian phytogeographic region. Anatomical structure of leaf trans-sections, dorsal epidermis and morphological variation of 23 accessions of *Phalaris minor* from different parts of Iran were studied. Coastal and inter-coastal characters were evaluated and the intra-specific variations were studied. Results indicate a lot of variations in shape and wall thickness of long cells, the number and type of short cell, stomata number per leaf area, shape of silica bodies, guard cells and the presence of different kind of hairs. Also sclerenchyma position, the shape of midrib and furrows of leaf blade and the features of vascular bundles show a lot of variations. Different accessions show variations in shoot length and spike shape and length. The results indicate that there may be some ecotypes in *Phalaris minor* of Iran. Statistical analysis is provided for Iranian populations of weedy *Phalaris minor*.

Key words: *Phalaris minor*, anatomical structure, morphological variation, Iran

INTRODUCTION

The genus *Phalaris* has had a complicated taxonomic and nomenclatural history (Baldini, 1995, 1993). *Phalaris* comprises 22 species of annual or perennial grasses in temperate regions of the world (Baldini, 1993). It is commonly adventive species of open habitats. *Phalaris minor*, an old world species of the genus, is an annual, Irano-touranian element with tetraploid ($2n = 28$) populations. It grows well in cultivated and waste places in different habitats of Iran (Bor, 1970). It is a weed of many winter season crops, including wheat and barley. Little canary grass is common in Eurasia, South and North America, East and South of Africa and Australia. Baldini (1995) found high variation in *Ph. minor* populations of Italy. Future crop improvements to annual canarygrass cultivars by either intra-specific or inter-specific hybridizations, or mutagenesis to introduce new traits, may benefit from a better understanding of the variability contained in annual *Phalaris* germplasm collections.

Ph. minor has both tillering and branching which gives it a rosette shape. It has fertile spikelets, hermaphrodite flower, with one sterile lemma. Its glume is 5-6 mm long with an erose wing. The seed of *Ph. minor* can be ground into a flour and used in making bread, cakes etc. It is rather small and fiddly to utilize. It is tolerant of most conditions, dry or wet. Seed colour varies from green (immature) to light yellow and glossy dark brown with hard seed coat. This species has an important weedy role and also in north east of Iran. It has been

studied and scientists have found the possible role of this as carcinogen in the occurrence of oesophageal cancer because of the presence of siliceous fibres in the seeds.

Matus and Hucl (1999) by studying isozyme variation within and among accessions of annual *Phalaris* species found that collections lack adequate genetic characterization for utilization. Among accessions polymorphism was greater than within-accession polymorphism in *Ph. minor*. Limited variation was detected by means of isozymes in annual *Phalaris* species.

Ph. minor is distinguished from other species of this genus in Iran by characters as spike shape, glum shape, colour and size, seed shape and size. Anatomical studies of different grasses in Iran show the diagnostic value of coastal and intercoastal features of epidermis and many other characters of transverse sections (Keshavarzi and Rahiminejad, 2005; Keshavarzi and Seifali, 2005; Keshavarzi *et al.*, 2002, 2004, 2005, 2007). This is the first study of *Phalaris* taxa in Iran. The purpose of this study is to evaluate the morphological and anatomical variation of different accessions of *Ph. minor* in Iran.

MATERIALS AND METHODS

In this study 17 populations of *Ph. minor* were gathered from 2004-2006 from different parts of Iran (Fig. 1 and Table 1). Anatomical observations were based on transvers sections and dorsal epidermis at the middle of leaf blade (2nd leaf below flag leaf). We use tissue removal and methyl green staining for study the



Fig. 1: Distribution map of sampled populations of *Ph. minor* in Iran

Table 1: Voucher details of *Ph. minor* sampled in this study

| Herbarium No. | Address | Collector |
|---------------|------------------------------------|-------------|
| 83m8 | Yazd, Ardakan, 1400 m | Keshavarzi |
| 68m12 | Khozestan, Mased Soleiman | Boromandi |
| 80m16 | Tehran, Chitgar Park | Keshavarzi |
| 83m14 | Yazd, Meyboud, 1400 m | Keshavarzi |
| 66m22 | Tehran, Saei Park, 1600 m | Keshavarzi |
| 71m17 | Qom, Qom | Vejdani |
| 70m18 | Mrkazi, Mahalat | Vejdani |
| 83m18 | Khozestan, Ramhormoz | Keshavarzi |
| 84m10 | Fars, Shiraz, 1500 m | Keshavarzi |
| 83m5 | Khozestan, Molasani | Farasat |
| 83m3 | Markazi, Save, Yalabad | Keshavarzi |
| 83m7 | Lorestan, Khoramabad, Kew | Keshavarzi |
| 83m19 | Khozestan, Shoush | Keshavarzi |
| 83m6 | Khozestan, Behbahan | Keshavarzi |
| 83m19 | Tehran, Vanak, 1700 m | Keshavarzi |
| 82m15 | Mazandaran, Sari | Komeilydost |
| 84m1 | Fars, Kazeroun, Baghe nazar, 900 m | Keshavarzi |
| 83m13 | Khozestan, Haftape, Choghazanbil | Keshavarzi |
| 80m23 | Golestan, Gorgan | Khaksar |
| 70m21 | Khorassan, 20 km to Gonabad | Khaksar |
| 83m24 | Khozestan, Dezful | Khaksar |
| 83m4 | Khozestan, Ahwaz | Keshavarzi |
| 72m11 | Semnan, Garmsar | Keshavarzi |

epidermis. Transverse sections were studied by double staining. Quantitative and qualitative anatomical characters are mentioned in Table 2 and 3. Morphological evaluation was based on quantitative (Table 4) and qualitative features. Morphological characters were measured and evaluated for all 10 individuals of each accession.

For grouping the accessions different methods of cluster analysis including single linkage, UPGMA and

Table 2: Studied anatomical characters of dorsal epidermis of *Phalaris minor*

| Quantitative characters | | | |
|-------------------------|-----------------------|----|----------------------|
| No. | Character | No | Character |
| 1 | Short Cell no. IC | 10 | Stomata length IC |
| 2 | Prickle No. IC | 11 | Stomata width IC |
| 3 | Macro hair IC | 12 | Stomata diameter IC |
| 4 | Stomata No.IC | 13 | Silica cell No. C |
| 5 | No of stomata band IC | 14 | Prickle No. C |
| 6 | Long cell length IC | 15 | Long cell Width C |
| 7 | Long cell width IC | 16 | Long cell Length C |
| 8 | Short cell length IC | 17 | Silica cell Width C |
| 9 | Short cell width IC | 18 | Silica cell Length C |

Qualitative characters

| No. | Character | No. | Character |
|-----|--|-----|---|
| 1 | Short cell (Single 0, single and twin 1) | 7 | Frequency of silica cell (Mild 2, numerous 1, few 0) |
| 2 | Frequency of short cell (Mild 2, numerous 1, few 0) | 8 | Prickle (present 1, absent 0) |
| 3 | Long cell width (Even 1, noneven 0) | 9 | Frequency of prickles (Few 0, numerous 1, Mild 2) |
| 4 | Thickening of long cell (Mild 2, thick 1, thin 0) | 10 | Macro hair (Present 1, absent 0) |
| 5 | Shape of long cell (Sinusian 1, even 0) | 11 | Shape of subsidiaries (Domes chapel, parallel sided 0) |
| 6 | Silica cell (Single 0, twin 1, both 2) | 12 | Silica cell walls (Curvaceous 1, no curvaceous 0) |

C = Coastal, IC = Inter-coastal

Ward as well as ordination base on Principal Component Analysis (PCA) were performed on standardized data. Squared Euclidean distance was used as a measure of similarity in cluster analysis. Uni-variate and multi-variate statistical analyses used SPSS ver. 14 software.

Table 3: Studied anatomical features (transverse section) of *Phalaris minor*

| Character No. | Character | State of character | Character No. | Character |
|---------------|------------------------------|---------------------------|---------------|-------------------------------------|
| 1 | Prickles | Presence 1 absence 0 | 9 | Number of Vascular bundles |
| 2 | Macrohair | Presence 1 absence 0 | 10 | Bulliform number |
| 3 | Differentiation of Mesophyll | Presence 1 absence 0 | 11 | Length of Buliform cells |
| 4 | Furrows at the back of leaf | Presence 1 absence 0 | 12 | Width of Buliform cells |
| 5 | Sclerenchyma Grider | Continuous 1 Disruptive 0 | 13 | Sclerenchym cell no at leaf margins |
| 6 | End of Leaf | Blunt 0 Pointed 1 | 14 | Inner Bundle sheath cell numbers |
| 7 | Leaf dorsal part | Smooth 1 Wavy 0 | 15 | Outer bundle sheath cell numbers |
| 8 | Outer Bundle sheath | Complete 1 Incomplete 0 | 16 | Adaxial Leaf prickles no |

Table 4: Studied quantitative morphological characters of *Phalaris minor* in Iran

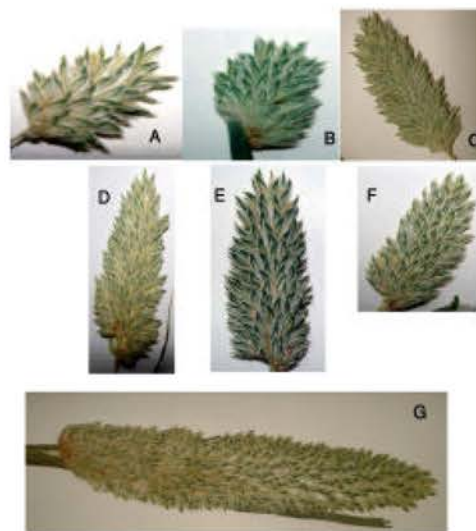
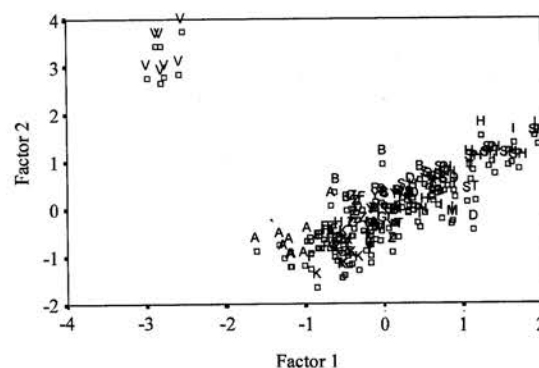
| No. | Character | No. | Character |
|-----|--------------------|-----|-----------------------------|
| 1 | Spike length | 11 | Length of longest internode |
| 2 | Spike width | 12 | Lemma width |
| 3 | Blade length | 13 | Palae length |
| 4 | Blade width | 14 | Palae width |
| 5 | Ligule length | 15 | Spikelets length |
| 6 | Upper glume length | 16 | Spikelets width |
| 7 | Upper glume width | 17 | Floret No. |
| 8 | Lower glume length | 18 | Culm length |
| 9 | Lower glume width | 19 | Leaf No. |
| 10 | Lemma length | | |

RESULTS

Morphological observation: A vast morphological variation was seen in spike features of *Ph. minor* accessions of Iran. Caryopsis is oval or elliptic, green pre-maturation and yellow to pale brown post-maturation. Qualitative morphological characters of spikes in populations of different parts of Iran show some variation in shape and compactness. There is a great homogeneity in other qualitative features. Compact spikes of this species are fusiform, cylindrical or oval in different accessions. Quantitative characters show a vast variation in spike length (Fig. 2).

Multivariate statistical analysis shows the diagnostic value of morphological characters in this species. Factor analysis of results shows that length of spike and upper glume are important factor of observed variation (Table 5). Two factors have the most important role in observed variations. First factor, it self causes the 35.2% of variation in *Ph. minor* populations of Iran. Characters as length of spike, upper glume, lemma, lower glume, width of spike and plant height are considered as first factor. 20% of variation is due to second factor which is related to width of upper glume and length of ligule. Standardized data were used for principal component analysis.

Principal component analysis shows that these accessions are closely related but some accessions of Vanak and Behbahan regions are somehow separate (Fig. 3).

Fig. 2: The spike morphological variation in different accessions of *Ph. minor* A) Meybod, B) Khoramabad, C) Ardakan, D) Mahalat, E) Yalabad, F) Ahwaz and G) BehbahanFig. 3: Principal component analysis of *Ph. minor* populations of Iran. V stands for Vanak (north of Tehran) and B for Behbahan (SW Iran) population

Anatomical observation: In abaxial leaf blade epidermis costal/inter-costal zonation is conspicuous. Long-cells of coastal and inter-coastal region are similar in shape and

the thickness of walls. Mid-inter-costal long-cells fusiform; have straight or only gently undulating walls. Stomata are common with (30-)33-39(-42) microns long and arranged in two rows. Subsidiaries are low dome-shaped, or parallel-sided. These cells show some variation between populations (Table 6). Inter-costal silica bodies when present rounded (oval). Costal silica bodies horizontally-elongated, crenate/sinuuous, smooth, rounded (or cuboid); occasionally cross or butterfly shaped. Papillae are absent. Short cells are single or twin in coastal region. These are arranged in 3-5 rows. In inter-coastal region these are few or absent.

Length of long and short cells and also stomata show variation in different populations. End walls of long cells are homogen and simple in Ardakan (Central Iran) population but in Meybod (Central Iran) and Tehran populations these are shortened and cells become fusiform (Fig. 4). Frequency of silica bodies in coastal and

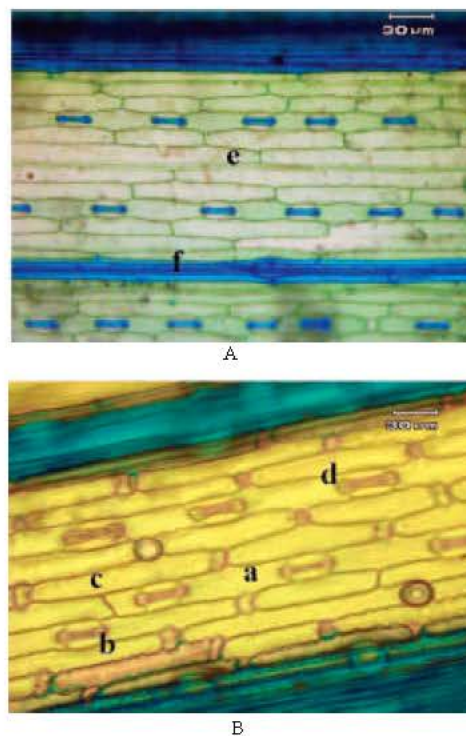


Fig. 4: Dorsal epidermis of *Ph. minor* population A) Baghe Nazar (Kazerun, S. Iran), B) Gonabad (NE. Iran) (a stands for short cell, b for silica bodies, c for long cell, d for subsidiaries, e for inter-coastal region and f for coastal region)

Table 5: Factor analysis in *Ph. minor* accessions

| Address character | Component | |
|--------------------|-----------|------|
| | 1 | 2 |
| Spike length | 0.84 | |
| Upper glume length | 0.82 | |
| Lemna length | 0.82 | |
| Lower glume length | 0.80 | |
| Spike width | 0.79 | |
| Plant height | 0.72 | |
| Spikelet width | 0.70 | |
| Upper glume width | | 0.82 |
| Ligules length | | 0.80 |

Table 6: Results of studied qualitative characters of dorsal epidermis in *Ph. minor* accessions

| Address character | Gaemshahr | Gonabad | Kazeroun | Ardakan | Behbahan | Khoramabad | Molasani | Sari | Shoush |
|--------------------------|-----------|---------|----------|---------|----------|------------|----------|------|--------|
| Short cell | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Frequency of short cells | 2 | 2 | 1 | 0 | 2 | 0 | 0 | 2 | 0 |
| Long cell width | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thickening of long cell | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| Shape of long Cell | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Silica cell | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Frequency of silica cell | 1 | 1 | 0 | - | 1 | - | 0 | 1 | 1 |
| Silica cell walls | 0 | 0 | 1 | - | 0 | - | 0 | 0 | 0 |
| Prickle | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| Frequency of prickles | 1 | 1 | 2 | 0 | 1 | 1 | - | 0 | 2 |
| Shape of subsidiaries | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Table 6: Continued

| Address character | Yalabad | Dezfoul | Garmsar | Majedsaleiman | Choghazanbil | Shiraz | Meybod | Mahalat | Tehran |
|--------------------------|---------|---------|---------|---------------|--------------|--------|--------|---------|--------|
| Short cell | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Frequency of short cells | - | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Long cell width | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thickening of long cell | 0 | 2 | 2 | 1 | 1 | 2 | 1 | 0 | 2 |
| Shape of long cell | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 |
| Silica cell | 0 | 0 | 1 | 0 | 0 | 0 | 0 | - | 1 |
| Frequency of silica cell | - | 1 | 2 | 1 | 1 | 1 | 0 | 0 | 2 |
| Silica cell walls | - | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Prickle | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| Frequency of prickles | - | 2 | 2 | 0 | - | 2 | - | 0 | 1 |
| Shape of subsidiaries | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |

Codes are mentioned in Table 2

inter-coastal regions is variable. Long cell walls of Gonabad population (NE. Iran) near coastal region are sinuate. By studying qualitative characters of dorsal epidermis it seems that width of short cells are the same in different populations. Silica bodies and short cells are single or twin. Short cells frequency is variable too. In Meybod and Molasani (SW Iran) populations prickles are not visible, but this kind of hairs is present in all other populations.

Considering quantitative features of dorsal epidermis we found that Garmsar population of *Ph. minor* has the most silica cells and Mahalat has the least. The least prickles were seen in populations of South west of Iran. Garmsar accession has the most prickles. The range of

prickles per leaf area was 1-12. Width and length of long and silica cells dose not show variation. Maximum stomata (10 and 12) per leaf area were seen in Garmsar and Sari accessions. In all studied populations two rows of stomata were seen in inter-coastal region of dorsal epidermis. The longest and largest stomata opening were seen in Garmsar and Sari populations of *Ph. minor* in Iran. The short cell number was not so varied in different accessions.

Transverse sections of leaf blade show that mesophyll has radiate or non-radiate chlorenchyma (Table 7). Leaf blade is adaxially flat (Behbahan population) or have dome shape or even pointed furrows (Fig. 5). Midrib has one bundle only. Bulliform cells

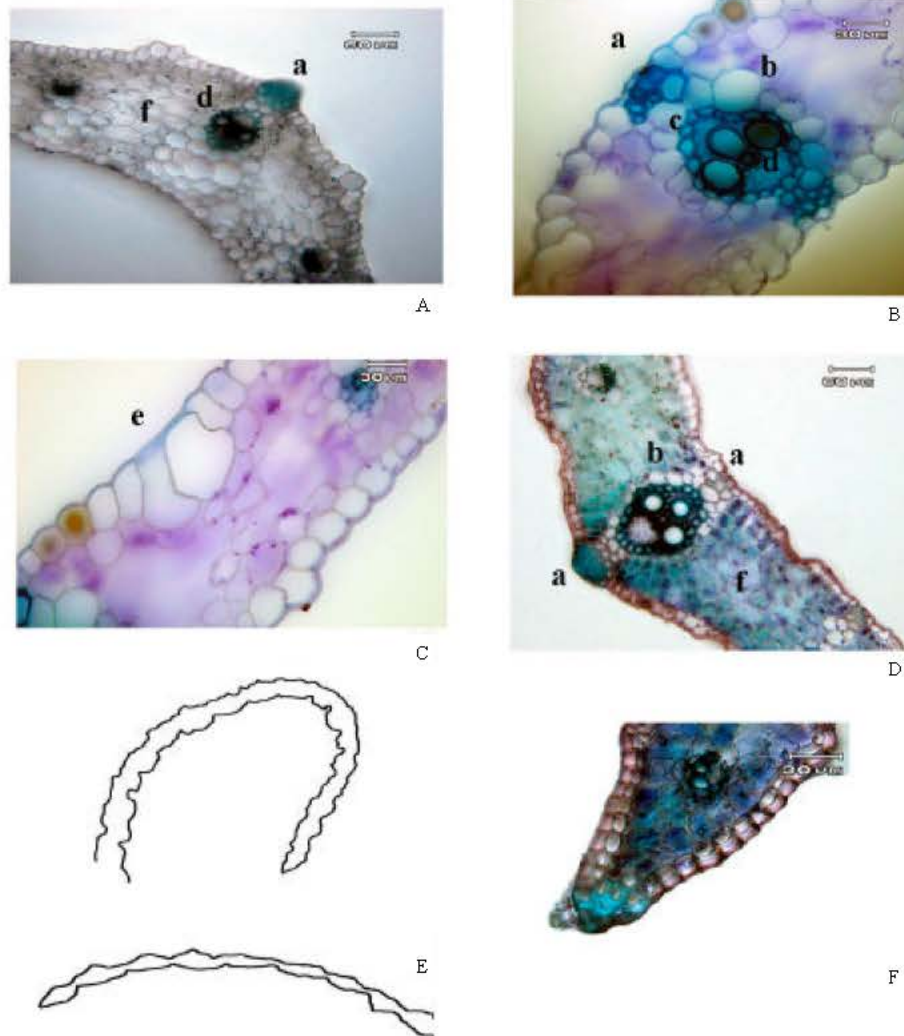


Fig. 5: Transverse section of *Ph. minor* population of A) Khoramabad (W. Iran), B and C) Gonabad (NE. Iran), D and F) Behbahan (SW. Iran), E) General shape of transvers section, Poulation of Top) Behbahan, Below) Baghe Nazar (Kazerun, S. Iran), (a stands for chlorenchyma continuum, b for outer and c for inner bundle sheath, d for vascular bundle, e for bulliform cells and f for the non-radiate mesophyll)

Table 7: Result of studied qualitative characters of leaf transvers sections in Ph. minor accessions of Iran

| Address character | Gaemshahr | Gonabad | Kaxeroun | Ardakan | Behbahan | Khoramabad | Molasani | Sari | Shoush |
|-----------------------------|-----------|---------|----------|---------|----------|------------|----------|------|--------|
| Prickles | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| Macrohair | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Differentiation of mesophyl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Furrows at the back of leaf | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Sclerenchyma girder | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| End of leaf | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| Leaf dorsal part | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Outer Bundle sheath | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |

Table 7: Continued

| Address character | Yalabad | Dezfoul | Garmsar | Masjedsoleiman | Shiraz | Meybod | Mahalat | Tehran |
|-----------------------------|---------|---------|---------|----------------|--------|--------|---------|--------|
| Prickles | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Macrohair | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Differentiation of mesophyl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Furrows at the back of leaf | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 |
| Sclerenchyma girder | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| End of leaf | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| Leaf dorsal part | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Outer Bundle sheath | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 |

present in discrete, regular adaxial groups; as simple fans. Each vascular bundle comprises two bundle sheaths (outer and inner). Both of them are complete in *Phalaris minor*. Outer bundle sheath has colourless cells which are connected to the sclerenchyma girder. All the vascular bundles accompanied by sclerenchyma. Combined sclerenchyma girders are sometimes present. Sclerenchyma always associated with vascular bundles.

Quantitative characters of leaf transvers sections show the highest number of buliform cells in Garmsar population.

DISCUSSION

By studying different qualitative and quantitative morphological characters it seems that the variation in size is related to its growing conditions. The size of culm is also very variable. The dimensions depend on edaphic conditions. In fact in eutrophic soils (cultivated fields) *Ph. minor* can be 100 cm tall and panicle 6 cm long whereas on oligotrophic soils the individuals are small, 10-15 cm tall with panicles up to 1.5 cm long. The upper margin of the glume is very variable from erose to entire. The incision of the glume margin is very variable. Sometimes this wide range of variation can be found in the same panicle. Many varieties have been based on this character but we think they are not worthy of taxonomic recognition. Other important features in this species are the dimension and number of the sterile lemmas. By studied morphological features the *Ph. minor* populations of Iran could be described as mentioned below:

Phalaris minor is an annual grass with 10-200 cm high purplish culm with hollow internodes. Leaf blades linear to lanceolate; broad, or narrow; 2-20 mm wide; flat;

persistent; dark green (immature) and rolled in bud. Ligule present and is an unfringed membrane; truncate, or not truncate; 2-12 mm long.

Inflorescence is a false spike, with spikelets on contracted axes, or paniculate; contracted in capitate form or more or less ovoid. In Iranian populations of this species different shape of compact spike cylindrical, oval and fusiform are observed. Spikelets pedicellate, 3.5-9.5 mm long; strongly compressed laterally; disarticulating above the glumes, or falling with the glumes. Rachilla terminated by a female-fertile floret. Glumes are two; more or less equal; long relative to the adjacent lemmas; pointed; awnless; carinate; with a conspicuously winged (1 nerved) keel which is similar and papery. Lower glume has 2 pale green nerves. Spikelets usually have fertile florets. The proximal lemmas are awnless and exceeded by the female-fertile lemmas. Female-fertile florets are 1(-2). Lemmas lanceolate to ovate, about 3 mm long, becoming indurated; entire; pointed; awnless; hairy, carinate; 5 nerved. Sterile lemma is solitary and about 1 mm long. Palea present; relatively long; entire to apically notched; awnless, thinner than the lemma to textured like the lemma; 1-nerved; keel-less. Lodicules are present; 2; free; membranous; glabrous; not toothed. Stamens are 3 and anthers 1.2-6 mm long. Ovary is glabrous, stigmas 2 and white. In all populations caryopsis is free from both lemma and palea; small and compressed laterally, oval or elliptic. Hilum is long-linear and the color of seeds are green (immature) and yellow and glossy dark brown with hard seed coat (maturity).

Anatomical variations of these accessions are in number of different cells per area and their shape. It seems that this wide phenotypic plasticity can not be recognized taxonomically. Statistical analysis shows that there may

be some ecotype formation of *Ph. minor* in Vanak region. There are also some anatomical variations in Garmsar population. It seems Garmsar and Vanak accessions should be treated genetically in future.

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