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Systematic Study of *Bromus danthoniae* (Poaceae) Native to Iran

M. Keshavarzi, S. Direkvandy and F. Abivardi
Department of Biology, Alzahra University, Vanak, Tehran, Iran

Abstract: In this study different varieties of *Bromus danthoniae* in Iran are considered. Qualitative and quantitative morphological characters were evaluated for 32 accessions. Epidermal features were also studied for these accessions. The most variable morphological characters in the species delimitation were also determined. The varieties show significant variations in features as Awn status, Spike and spikelet length, Nerve number of Upper Glume, Lemma, Palea and Caryopsis length. Diagnostic features based on studied characters are identified and the varieties relationships are discussed.

Key words: *Bromus danthoniae*, morphology, varieties

INTRODUCTION

Bromus L. (Poaceae) with 35 annual and perennial species in Iran, has been traditionally divided to some sections due to its morphological differences. *B. danthoniae* (Neobromus section) has not been studied properly. There are three varieties of this species in Iran Platue (Bor, 1968, 1970) var. *danthoniae*, var. *lanuginosus* and var. *uniaristatus* and merely one variety in Iran (var. *danthoniae*). *Bromus danthoniae* Trin. ex C.A.Mey. var. *uniaristata* Melderis was recently recorded in this study from Iran.

Bromus danthoniae is an annual species with three awned lemmas. This species comprise an ancestral

position in this genus (Oja and Jaaska, 1998). Recent molecular study supports the insertion of *B. danthoniae* in *Bromus* section. In this study intra-specific variations in *B. danthoniae* accessions of Iran is studied for the first time. The variety limitations is evaluated and an efficient identification key is provided.

MATERIALS AND METHODS

Bromus danthoniae accessions were gathered from different localities from 2005 till 2008 in Iran (Table 1, Fig. 1). Ten individuals of each accession were studied. Totally 67 morphological characters were considered by studying literature and the filed observation



Fig. 1: Map of studied accession of *Bromus danthoniae* varieties in this study

Table 1: Voucher details of *Bromus danthoniae* sampled in this study

Taxon	Locality	Herbarium No.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, S. Chenaran, Farizi, Dahaneh Jaji, 1890- 2060 m	36362FUMH
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Neyshabour, Attaie, 1550 m	8514n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Mashhad, Akhlamad Village, 1500 m	8513n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Mashhad, Torogh Dam, 1200 m	843n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, S Chenaran, 3 km to Kandelan, 1832 m	36081AFUMH
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Mashhad to Kalat, 1100 m	8517n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, S Chenaran, between Farizi & Abneh, 1600 m	3599FUMH
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, S Chenaran, 3 km Farizi to Dehsorkhe, 1720-1810 m	36598FUMH
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Mashhad, Ferdousi University, Pardis, 1050 m	832n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Bojnourd, Beshghardash, 2200 m	8516n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Sabzevar, Sange Sefid Village	8518n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, Mashhad to Shandiz	8515n.
<i>B. danthoniae</i> var. <i>danthoniae</i>	Khorassan, E Torbat Heydarieh, 1250 m	8536n.
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, Khoramabad to Kouhdasht, Chegeni, 35 km SW Khoramabad	874d
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, 5 km Boroujerd, Sarabezarem Village	873d
<i>B. danthoniae</i> var. <i>danthoniae</i>	Lorestan, 5 km Boroujerd, Sarabezarem Village	873d
<i>B. danthoniae</i> var. <i>uniaristatus</i>	Lorestan, 5 km Boroujerd, Sarabezarem Village	873d
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, 40 km S Khoramabad, Dashte Dadabad	879d2
<i>B. danthoniae</i> var. <i>uniaristatus</i>	Lorestan, 40 km S Khoramabad, Dashte Dadabad.	879d
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, 15 km SW Khoramabad, Dashte Parsilon	878d
<i>B. danthoniae</i> var. <i>danthoniae</i>	Lorestan, 15 km SW Khoramabad, Dashte Parsilon	878d2
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, 30 km SW Khoramabad, Babaabas Village	877d
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, Khoramabad to Andimeshk, 10 km to Khoramabad, Miangelal Village	876d
<i>B. danthoniae</i> var. <i>lanuginosus</i>	Lorestan, 35 km S Khoramabad, Gosheh Village	8714d
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Ghochak	871mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, 20 km to Firouzkuh	872mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Shemshak	8713mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Shemshak	8713mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Robatkarim, Roudeshour basin	8712mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Karaj	8711mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Tehran	8710mk
<i>B. danthoniae</i> var. <i>danthoniae</i>	Tehran, Vanak	875mk

Table 2: Studied quantitative morphological characters in studied varieties

No.	Characters	No.	Characters
1	Spikelet length excluding awn	17	Lemma awn length
2	Spike length	18	Lemma nerve No.
3	Spike width	19	Palea length
4	Leaf blade length	20	Palea width
5	Leaf blade width	21	Palea nerve No.
6	Length of longest internodes	22	Length of inflorescence stalk
7	Spikelet length	23	Caryopsis length
8	Spikelet width	24	Caryopsis width
9	Upper Glum length	25	Floret number
10	Upper Glume width	26	Sterile flower No.
11	Upper Glum nerve No.	27	Fertile floret No.
12	Lower Glume length	28	Lemma awn No.
13	Lower Glume width	29	Culm length
14	Lower Glume nerve No.	30	Anther length
15	Lemma length	31	Length of Palea Gap
16	Lemma width	32	Length of caryopsis corona

All measured characters are in mm

(Table 2, 3). All studied materials are deposited in Alzahra University, Iran. In dorsal epidermis of flag leaf in *Bromus danthoniae* accessions, 34 qualitative and quantitative characters were studied (Table 4, 5). In order to detect significant differences in studied characters among populations of each species and also among different varieties, Analysis of variance (ANOVA) followed by the Least Significant Differences (LSD) tests were performed. To reveal taxa relationships, cluster analysis and Principal

Component Analysis (PCA) were used. For multivariate analysis the mean of quantitative characters were used while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. The average taxonomic distances and squared euclidean distances were used as dissimilarity coefficient in cluster analysis of morphological data. In order to determine the most variable morphological characters among the species

Table 3: Studied qualitative morphological characters in *Bromus danthoniae* varieties

Characters	States of character
Age of plant	Annual 0, perennial 1
Spike shape	Connate 0, obovate 1, oblong or elliptic 2
Spike curved	Unilateral 0, bilateral 1, none of them 2
Spike cover	Glabrous 0, pilose 1
Spike sort sitting on stem	Straight 0, recurved 1
Spike density	Densa 0, effuse 1
Glum color	Light green 0, deep green 1
Glum apex	Acute 0, rounded 1
Glum cover	Glabrous 0, pilose 1
Glum hair density	Dense 0, effuse 1
Lemma apex	Unbifid 0, bifid 1 a little bifid 2
Lemma tissue	Without nerve 0, nervy 1
Lemma cover	Glabrous 0, pilose 1, hirtellous 2
Lemma nerve convergence	Present 0, absent 1,
Lemma hair density	Densa 0, effuse 1
Lemma shape	Uniawn 0, tri-awn 1, none of them 2
Palea tissue	Without nerve 0, nervy 1
Palea nerve convergence	Present 0, absent 1
Grain color	Deep yellow 0, brown 1, green 2
Grain shape	Tall elliptic 0, rounded 1
Awn	Straight 0, recurved 1
Inflorescence	Fragile 0, strict 1 Rachilla
Glum rachilla	Connate 0, nonconnate 1
Leaf blade hair	Glabrous 0, pilose 1
Lemma awn color	Green 0, purple 1
Ligule apex shape	Without teeth 0, short toothed 1, deep toothed 2
Stem status	Erect 0, curvature in first node 1, very curved 2
Stem cover	Hirtellous 0, glabrous 1, pilose 2
Stem cover at inflorescence	Pilose 0, glabrous 1
Sheaths cover	Glabrous 0, pilose 1
Pedicel shape	Strict 0, wavy-hairy 1, tall-hairy 2, none of them 3
Palea margin shape	Ciliate 0, hairy 1, none of them 2
Glum texture	Scarious 0, coriaceous 1
Lemma texture	Scarious 0, coriaceous 1
Status of hair in node	Pilose 0, glabrous 1

Table 4: Quantitative studied character in dorsal epidermis of *Bromus danthoniae*

No.	Characters
1	Inter-coastal zone
2	Short cell No.
3	Prickle No.
4	Macro hair No.
5	Stomata No.
6	Stomata row No.
7	Long cell length
8	Long cell width
9	Short cell length
10	Short cell width
11	Coastal zone
12	Silica bodies No.
13	Prickles No.
14	Macro hair No.
15	Long cell length
16	Long cell width
17	Length of silica cells
18	Width of silica cells

studied, factor analysis based on principal components analysis was performed. SPSS ver. 15 (2006) was used for statistical analysis.

Table 5: Qualitative studied characters in *Bromus danthoniae* accession in Iran

No.	Characters and states of characters
1	Short cell (single+, single and twin+-)
2	Amount of short cell (high+, middle+-, low-)
3	Long cell width (monotonous+, non monotonous-)
4	Long cell wall thickness (high+, middle+-, low-)
5	Long cell wall form (sinusoid+, smooth-, pity+-)
6	Silica bodies (single-, twin and single+)
7	Amount of silica bodies (high+, middle+-, low-)
8	Prickle (existence+, absence-)
9	Amount of prickle (high+, middle+-, low-)
10	Macro hair (existence+, absence-)
11	Amount of macro hair (high+, middle+-, low-)
12	Stomata companion cell form (dampy+, parallel-, none of them+-)
13	Silica bodies form (rectangular+, nonrectangular-)
14	Silica bodies wall (wavy+, none wavy-)
15	Short cell form (rectangular+, nonrectangular-, rectangular and nonrectangular+-)

RESULTS AND DISCUSSION

Micro morphological observations: Dorsal epidermis of *B. danthoniae* var. *danthoniae* reveals that in coastal zone there are silica bodies and long cells. Long cells walls are smooth and thin, their width is not same. Silica cells are single or twine and with high frequency (8-12 per leaf area). No prickles or macro hair is visible here. Inter-coastal region is characterized by long cell with smooth cell wall and even width. Short cells are single and with medium frequency in all varieties. Stomata are observed in 2 to 4 rows. Subsidiaries are parallel sided to dome shaped. Prickles are distributed in low frequency but macro hairs with highest frequency (Fig. 2A, B). There are some modifications in *B. danthoniae* var. *lanuginosus* and var. *uniaristatus*. In var. *lanuginosus* there are up to 6 rows of stomata while there are only two in var. *uniaristatus*. Subsidiaries are dome shaped in var. *lanuginosus* and parallel sided in var. *uniaristatus* (Fig. 3A-D).

Morphological observations: Evaluating qualitative morphological characters shows some how continuous range of variation. Quantitative features are varied due to different varieties. Lemma awn number is directly related to the variety. Length of Palea gap, Anther length, corona length of Caryopsis, Palea width and Caryopsis width do not show much variation. Analysis of variance shows that except Spikelet width, all studied features are significantly varied in these 3 varieties. The mean of such characters may be useful for the infra-specific delimitations. This is supported by the clustering (Fig. 4) of the studied varieties. In Fig. 4 it is evident that *Bromus danthoniae* var. *danthoniae* and *B. danthoniae* var. *lanuginosus* are clearly separated from each other. The cluster analysis of 3 varieties of *Bromus danthoniae* of Iran, based on quantitative and qualitative morphological

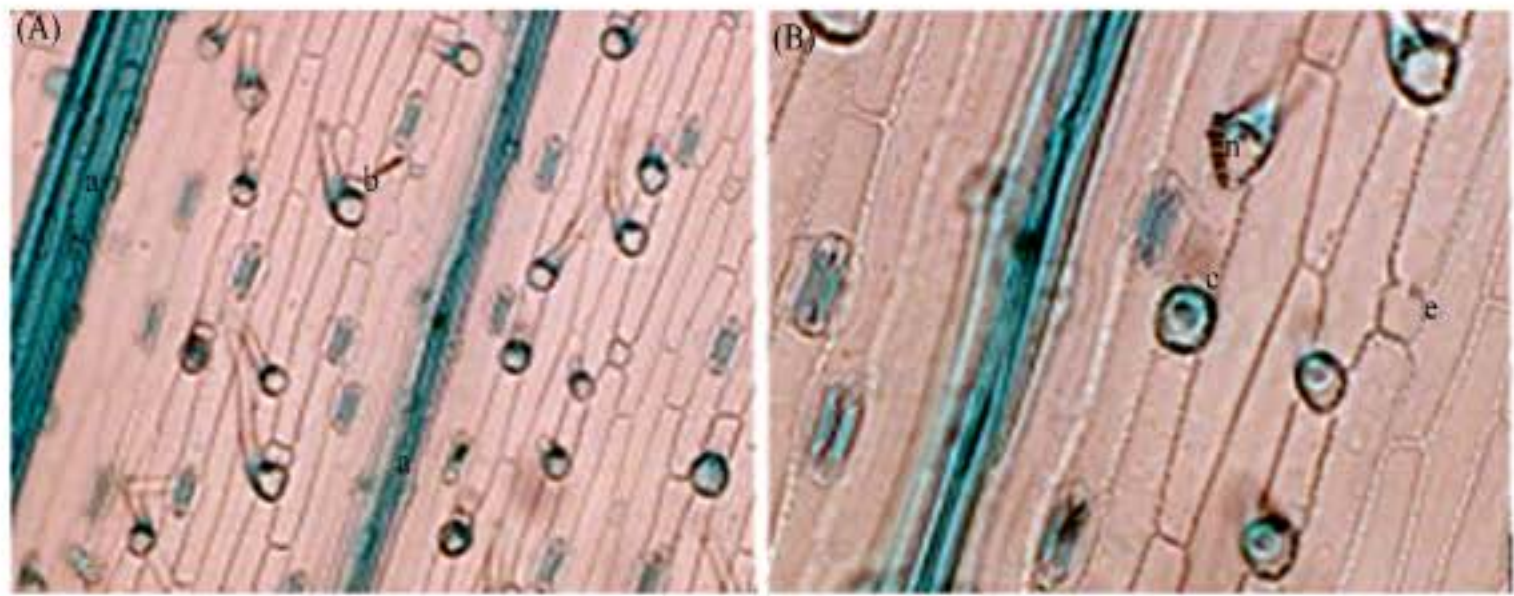


Fig. 2: Dorsal epidermis of *B. danthoniae*. (A) Farizi population and (B) pardis population, a: Coastal, b: Inter-costal zone, c: Long cell, e: Shoer cell, h: Macro hair

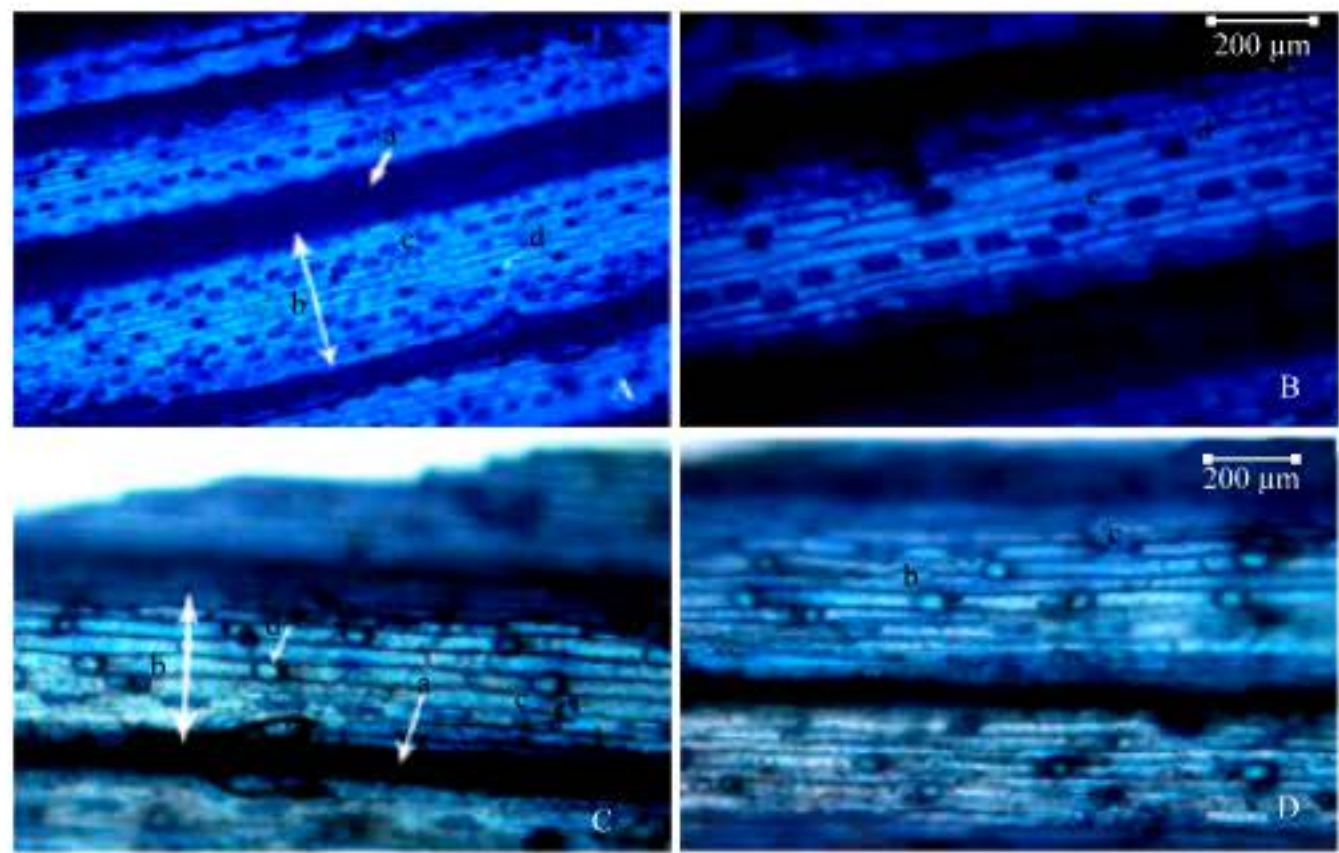


Fig. 3: Dorsal epidermis of *B. danthoniae* var. *lanuginosus* (A, B) Roudeshour population), *B. danthoniae* var. *uniaristatus* (C, D) Shemshak population). a: Coastal zone, b: Inter-coastal zone, c: Stomata, d: Short cell

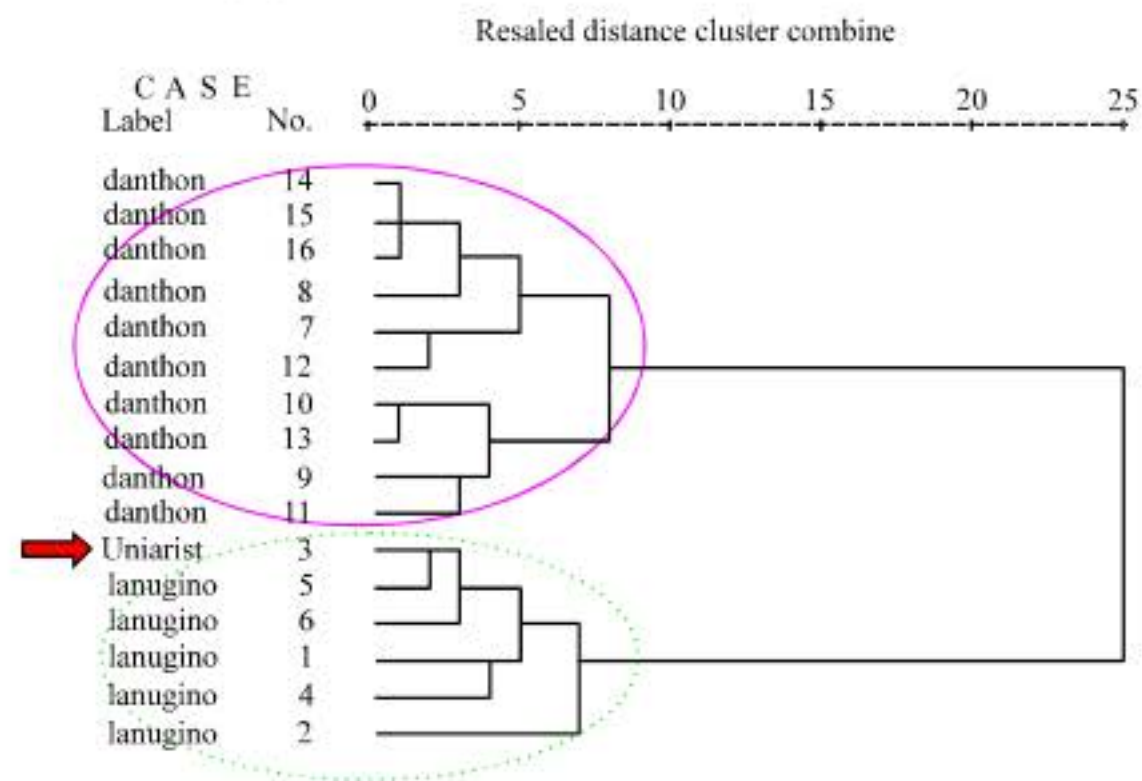


Fig. 4: WARD clustering of *Bromus danthoniae* based on mean of morphological characters

Table 6: Factor analysis results based on morphological characters for *Bromus danthoniae* populations of Iran

Characters	Factor		
	1st	2nd	3rd
Awn status	0.91		
Spikelet length	0.883		
Spike length excluding awn	0.863		
Upper glume nerve No.	0.817		
Lemma cover	0.805		
Spike length including awn	0.803		
Lemma length		0.731	
Palea length		0.693	
Caryopsis length			0.654

characters shows two major clusters. The first major cluster is comprised of two sub-clusters. As it is evident these two varieties are closely related.

Var. *uniaristatus* is nested in var. *lanuginosus* clusters (red arrow in Fig. 4), but due to the low number of individuals and populations the final deduction about their relationship is not accurate.

In order to determine the most variable characters among the studied species, factor analysis based on PCA was performed revealing that the first three factors comprise about 50% of total variation. In the first factor with about 30% of total variation (Table 6), characters such as Awn status, Spike length excluding awn, Upper Glume Nerve number, Lemma cover and Spike length including awn possessed the highest positive correlation (≥ 0.7). In the second factor with about 10% of total variation, characters as Lemma length and Palea length possessed the highest positive correlations. Third factor causes almost 7% of total variation due to the variation in caryopsis length. So, these are the most variable morphological characters among *Bromus danthoniae* varieties of Iran.

DISCUSSION

Elements of *Neobromus* section are clearly defined with their pointed Glume which has a gap and 3-awned lemmas. Based on karyological data, Stebbins (1981) suggested an origin in the Tertiary in Eurasia for the genus *Bromus*. Stebbins (1981) considered that the most primitive, now extinct, *Bromus* species probably differentiated in south-west Asia during the Miocene. Oja (1998) believed that central and western Europe, Mediterranean region and near Middle East is the modern and paleo center of evolution for *Bromus* species. Iran which is located in Middle East is one of active regions in *Bromus* diversification. *Bromus danthoniae* is one of the most variable grasses of Iran. This taxon has lot of variations in its vegetative and reproductive parts. A vast range of hair cover and spike color is observed in *Bromus danthoniae* populations. This plant, being a dwarf grass

of desert lands, show great patches of vegetation in cultivated and wetlands. This study shows that 3 awned lemma is one of the best features in separation of its infra-specific variations in Iran. At maturity individuals show curved awns. Aryavand (2002) found that morphology of panicles and to a lesser extent, of spikelets can be greatly affected by the water and soil nutrient supply. Some characters vary considerably between juvenile and mature states.

There are 3 distinct varieties for this species in Iran. One of them is recorded for the first time in this study. Due to the variation in soil moisture and texture it seems that these are making different locally adapted ecotypes in Iran. *Bromus danthoniae* could be described as: Annual plants, Culms erect, or geniculately ascending; 5-50 cm long. Leaf-sheaths pubescent or glabrous. Ligule an eciliate membrane. Leaf-blades 10-15 cm long; 2-4 mm wide. Both leaf-blade surfaces pubescent; may loss these after maturity.

Inflorescence a compact, ovoid panicle. 5-10 cm long; 1-5 cm wide. Panicle branches shorter than spikelets. scabrous. Comprising 8-16 fertile florets; Spikelets elliptic; laterally compressed; 6-12 mm long; 40-20 mm wide; Glumes persistent; dissimilar; Lower glume lanceolate; 5.5-8 mm long; 0.8-0.9 times length of upper Glume; Chartaceous; Without keels; 3-5-veined. Upper Glume elliptic; 7-9 mm long; 7-9-veined. Upper Glume apex acute. Lowest fertile elliptic; 8-12 mm long; Chartaceous; much thinner on margins; 9-11-veined. Lemma surface glabrous, pubescent, pilose, or villous. Lemma apex dentate; 2-fid; awned; 3-awned. Lower lemma seldom 1-awned, but upper lemmas 3-awned. Principal lemma awn dorsal; arising 2-4 mm under tip of back of lemma. curved; spreading; central awn 15-25 mm, lateral ones arising dorsally; 4-10 mm length. Palea shorter than the lemma, ciliate on the keels; anthers 1-1.8 mm long. Caryopsis with adherent pericarp; hairy at apex; apex fleshy. Hilum linear.

Identification key for varieties:

- Spikelets glabrous; lateral awn conspicuous
var. *danthoniae*
- Spikelets hairy 2
- Leaf sheath without hair, lower lemma 3-awned
var. *lanuginosus*
- Leaf sheath hairy, lower lemma 1-awned
var. *uniaristatus*

var. *danthoniae*

Inflorescence without hairs: These are distributed in habitats less than 2200 m from sea level in Iran. We visit this variety in different parts of Iran:

Khorassan: Torbat Heydariyeh, Neyshabour mountain, Akhlamad fall, Kalat Village, etc.

var. lanuginosus

Spikelets with hairs: This variety is found in open lands of up to 3000 m from sea level in Iran.

This variety was previously recorded from Iraq. In this study it is recorded for the flora of Iran for the first time. We found these populations from Roudeshour region and Vanak Village both in Tehran.

var. uniaristatus: With short hairs, upper lemma 3-awned, others 1-awned, glumes and lemmas membranous.

This variety is found in different habitats of Iran up to 2000 m from sea level. This taxon was previously recorded from Iraq. This variety was recorded for the first time from Iran by Keshavarzi *et al.* (2007). In this study we recorded this again from Lorestan: Sarabe Zarem, Tehran: Shemshak and Khorassan: North of Mashhad, Torbatejam, Torbate Heydarieh, Khaf, Gonabad and Kadkan altitudes.

Scholz (1998) believed that in the Near-Middle East region many intermediate populations exist between *B. pseudodanthoniae* and *B. danthoniae*. Some intermediate were found between its varieties itself. Studied materials confirmed that more attention should pay to the previous speculations of the hybrid formation between *B. lanceolatus* and *B. danthoniae*. It seems that to solve their taxonomic problems at first we should consider polymorphism in *B. danthoniae*. Present observations revealed that the range of variation in this species is more than expected. Further studies should

especially Irano-Turanian region has been the center of consider intermediate populations cytogenetically. In conclusion, it is suggested that the Southwest Asia, diversity of the sect. *Neobromus*. It seems that like *Genea* section, center of origin, diversification and greatest ecological range for *Neobromus* section is in Southwest Asia.

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