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Morpho-phenology and chromosome number of Iranian *Bromus danthoniae* Trin. genotypes

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ABSTRACT- *Bromus danthoniae* Trin. is an annual grass species which grows mainly on dry grassy rocky mountain slopes and grassy steppe, and is grazed by many herbivores and recognized as a useful pasture plant. The chromosome number, morphological and anatomical traits of 82 genotypes of *B. danthoniae* belonging to three sub-taxa were investigated. Twenty-seven quantitative and 20 qualitative morphological traits were evaluated. The results of analysis of variance showed that *B. danthoniae* genotypes varied significantly for all quantitatively tested traits. Based on cluster analysis, the genotypes were divided into four groups which mostly corresponded to their subspecies identities. According to correlation analysis, lemma length had strong positive correlations with other traits such as awn length (r=0.55**), lemma width (r=0.72**) and caryopsis length (r=0.84**). In addition, lemma width was significantly correlated with the traits like floret number per spikelet (r=0.47**) and caryopsis length (r=0.58**). Based on factor analysis, the first three factors encompass about 51% of total variation. Overall, lemma length and lemma width were the two reliable traits for morphological investigations in this species. The results of chromosome counting showed that *B. danthoniae* subsp. *danthoniae* and *B. danthoniae* var. *lanuginusos* Roshev. were diploid (2n=2x=14) whereas *B. danthoniae* subsp. *pseudodanthoniae* (Drobov) H. Scholz was tetraploid (2n=4x=28). The results of leaf surface anatomy showed that tetraploid genotypes had larger stomata but lower stomatal density than the diploid ones, thus a comprehensive relationship between genome size and guard cell size cannot be ruled out as a plausible explanation for differentiating the ploidy levels. The high morphological variations among the *B. danthoniae* genotypes explain the habitat distribution of this species and enable us to incorporate this knowledge into practice by exploiting the variation for improvement of pasture production.

INTRODUCTION

Bromus L. (Poaceae, Pooideae) is a plant genus which comprises approximately 150 C3 grass species (Watson and Dallwitz, 1992), of which 39 species and 40 taxa were found in Iran (Naderi and Rahiminejad, 2015). It is morphologically a complex genus because many species of this genus are difficult to identify (Saarela et al., 2007). *B. danthoniae* Trin. is one of the important species of the genus which is mainly Irano-Turanian, largely distributed in the steppes and hammadas ecosystems in the West Mediterranean area and extends to Himalaya (Kamari et al., 1998). This is an annual grass species that comprises the original ancestral genome in the genus *Bromus* (Oja and Jaaska, 1998). *B. danthoniae* grows mainly on dry grassy rocky mountain slopes, mountain steppe and grassy steppe in the plains. This species is also found abundantly in the dry grassy valleys and is grazed by many herbivores and recognized as a useful pasture plant (Townsend and Guest, 1968). While the importance of *B. danthoniae* species needs to be acknowledged for its role in the sustainable grassland production due to its high environmental adaptation and for its important role in preventing soil erosion as well as grassland degeneration and desertification, little information is available on the genetic diversity in this species.

Bor (1970) reported the first revisionary treatment of southwest Asian *Bromus* species, and incorporated the species present in Iran and neighboring countries. He divided *B. danthoniae* into three varieties, some of which were later named as subspecies (Scholz, 1998). Hence forth several other studies were published on intra-species classification (taxonomy) of the *B. danthoniae* (Clayton et al., 2006; Hamzehee et al., 2007; Valdés et al., 2009; Memariani et al., 2012; Naderi and Rahiminejad, 2015). According to Euro Med checklist, *B. danthoniae* includes two subspecies *danthoniae* and *pseudodanthoniae* (Valdés et al., 2009). In the current study, 82 *B. danthoniae* genotypes belonging to subsp. *danthoniae,* and subsp. *pseudodanthoniae* (Drobov) H. Scholz. as well as var. *lanuginusos* Roshev. were used, which allowed us to investigate intra specific variations. In addition, the germplasm exploration has been carried

out in four West Iran provinces stretching from Uremia (West Azerbaijan, northwest) to Mehran (Ilam, southwest) which encompass various climatic conditions. This shows that *B. danthoniae* is well adapted to the harsh climatic conditions including temperatures (low/high), dry and salinity (Arzani and Ashraf, 2016). Our recent work shows that the *B. danthoniae* genotypes originated from Uremia Salt Lake shore can tolerate high-salinity conditions (350 mM NaCl) (Rezaei et al., 2017). Nonetheless, the strong adaptation potential of this grass species has yet to be fully exploited.

Morphological studies have been performed in *Bromus* spp. in regionally and geographically distinct locations such as: Argentina (Aulicino and Arturi, 2008), Central Europe (Koch et al., 2016), North America (Novak and Mack, 2016), Poland (Skrajna et al., 2012) and China (Feng et al., 2011). In addition, cytological studies were conducted in this genus including Sheidai and Nourozi (2005), Sheidai and Fadaei (2005), Mirzaie-Nodoushan et al. (2006) and Klos et al. (2009). In plants, polyploidy is a key element in evolution and direct relationship between adaptation and genome size may justify the huge variations observed in the DNA content of various plant species (Arzani and Ashraf, 2016). With the increasing number of gene copies, the plant enables to harbor a new favorable mutation which facilitates genetic flexibility and promotes adaptive evolution (Sattler et al., 2016). Among the major consequences, the ‘‘gigas’’ effect (increase in cell size and in turn plant organs) and buffering of deleterious mutations are the major polyploidy features that plant breeders try to exploit and manipulate to improve crop performance (Hegarty et al., 2013). A strong relation between ploidy and cell size is found consistently in the plant kingdoms (Lomax et al., 2014). In particular, the sizes of the stomata guard cells and other cell types in leaf are associated with the size of genome (ploidy level) in the angiosperms (Beaulieu et al., 2008). Accordingly, Beck et al. (2003) demonstrated the length and frequency of the stomata can be used to indirectly detect the polyploidy level of plants.

Despite the potential contribution of *B. danthoniae* to sustainable grassland production (Rezaei et al., 2017), there is limited information concerning the morpho-phenology and leaf anatomy variations in this species. Therefore, the objectives of the present investigation were to assess the inter and intra-specific variations in morphological, phenological, chromosome number and leaf surface anatomical traits in *B. danthoniae* genotypes collected from West of Iran.

MATERIALS AND METHODS

Plant Materials

Eighty-two genotypes of *Bromus danthoniae*, including 72 genotypes from the subspecies *danthoniae*, three genotypes from the subspecies *pseudodanthoniae* and seven genotypes from the variety *lanuginusos* collected from different locations in West of Iran were used in this study (Supplementary Table S1). Collected seeds from individual plants were taken, and subsequently grown in separate rows under field conditions in the growing season 2014–2015 at Isfahan University of Technology.

A randomized complete block design with two replications was used in this study. Each plot consisted of two rows with 1.5 m long and spaced 30 cm apart. The soils at the experimental field are Typic Haplargids of the arid tropics with a silty clay loam texture. Mean annual precipitation and temperature at this site are 149 mm and 15.4°C, respectively.

Morphological Traits

Twenty qualitative (Table 1) and 27 quantitative traits (Table 2) were measured based on IPGRI descriptor (International Plant Genetic Resource Institute) and previous morphological studies in *Bromus*. Each quantitative piece of data was recorded by the mean of eight measurements for each plot of the experiment (totally 164 plots).

Chromosome Counting

Chromosome counting was performed using 15 selected genotypes (out of 72) from the subspecies *danthoniae*, three genotypes from the subspecies *pseudodanthoniae* and seven genotypes from variety *lanuginusos* (totally 25 genotypes). Briefly, root tip meristems obtained from seedlings were pre-treated in mono-brome-naphthalene, fixed in a 1:1 (v/v) solution of 10% (v/v) formalin and 1% (w/v) chromic acid, hydrolyzed with NaOH and then stained with hematoxilin solution. After removing the root cap from well-stained root tips, the meristem cells were squashed in 45% (v/v) acetic acid (according to Agayev (2002) with some modifications) and used for cytological observation. The images were taken using a light microscope (Nikon Eclipse E600, Tokyo, Japan) and captured by Photograb 300 software version 2.1 (Fujix TM 300 Z sh-3z, Fuji, Japan).

Anatomical Analysis

For stomatal and epidermal cell countings, a clear nail varnish was applied on the adaxial surface of leaves from two genotypes of each sub-taxa (totally six genotypes) to obtain an epidermal impression. Then, the samples were mounted on glass slide, covered with a cover slip and observed under a light microscope (Nikon Eclipse E600, Tokyo, Japan). Image measurements were taken using TsView 7.1 software (Tucsen, Fuzhou, China) and were counted from eight microscope fields of view randomly selected at a magnification of x 200 (stomatal countings and epidermal cell length) and x 1000 (stomatal length). Stomata density was calculated by converting the number of stomata per field into the number of stomata mm^{-2}.

Statistical Analysis

Shannon index (Shannon and Weaver, 1998) was calculated to evaluate the diversity of qualitative traits by the software PopGene2. Analysis of variance (ANOVA) was carried out using PROC GLM using the software SAS version 9.3.

Table 1. Measured qualitative traits, their descriptions and Shannon index in *B. danthoniae* genotypes

No.	Trait	States of trait	Shannon index
1	Spike shape	Connate 1, obovate 2, oblong or elliptic 3	0.79
2	Spike cover	Glabrous 1, pilose 2	0.53
3	Spike density	Densa 1, effuse2	0.55
4	Glum apex	Acute 1, rounded 2	0.68
5	Glum cover	No pubescence 1, light pubes. 3, heavy pubes. 5	0.41
6	Lemma apex	Unbifid 1, bifid 3, a little bifid 5	0.49
7	Lemma cover	No pubescence 1, light pubes. 3, heavy pubes. 5	0.87
8	Lemma shape	Uniawn 1, tri-awn 2	0.11
9	Lemma texture	Scarious 1, coriaceous 2	0.11
10	Grain color	Yellow 1, brown 2, green 3	0.68
11	Grain shape	Rounded 1, Tall elliptic 2	0.69
12	Awn	Straight 1, recurved 2	0.65
13	Awn color	Yellow 1, purple 2	0.42
14	Stem cover	No pubescence 1, light pubes. 3, heavy pubes. 5	0.94
15	Stem cover at inflorescence	No pubescence 1, light pubes. 3, heavy pubes. 5	0.92
16	Hair in node	No hair 1, hairy 2	0.69
17	Leaf cover	No pubescence 1, light pubes. 3, heavy pubes. 5	0.44
18	Sheat cover	No pubescence 1, light pubes. 3, heavy pubes. 5	0.69
19	Anther color	Yellow 1, purple 2	0.42
20	Stem form	Upright 1, half upright 3, prostrate 5	0.96

To contrast different groups of genotypes, orthogonal contrasts were made between diploid genotypes vs. tetraploid genotypes for anatomical traits using "contrast option" of PROC GLM, by software SAS version 9.3 (SAS Institute, 2011). Cluster analysis was performed with the standardized mean of quantitative traits while the qualitative traits were coded as binary/multistate traits (Table 1). Ward's method of cluster analysis was employed using a measure of similarity (squared Euclidean distance) by the STATGRAPHICS Centurion XVI.I software. To determine the reasonable number of the clusters, the agglomeration distance plot was used. In addition, factor analysis was carried out on quantitative traits based on PCA, according to Johnson and Wichern (2007). The relationships between quantitative traits as well as the relationships between phenological traits and geographical coordinates of the origins of collected genotypes were determined by correlation analysis using SPSS software version 17.0.

RESULTS AND DISCUSSION

The calculated Shannon index values for qualitative traits showed that stem form, stem cover and stem cover at inflorescence (with H=0.96, 0.94 and 0.92, respectively) had the highest values, and lemma shape and lemma texture (both with H=0.11) had the lowest values of this index among qualitatively tested traits in *Bromus danthoniae* genotypes.

The results of ANOVA showed that studied genotypes varied significantly for all quantitative traits (Table 2). Descriptive statistics (mean, minimum, maximum, variance and coefficient of variation) of quantitative morphological traits in the studied genotypes were shown in Table 3. The phenological traits (days to flowering, days to anthesis and days to maturity) had the lowest variation (CV= 3.26, 3.09 and 2.87, respectively), while tiller number, length of spikelet stalk, spikelet number per spike and Spike width (CV= 73.49, 39.91, 37.85 and 37.15, respectively) had the highest variation in the studied genotypes. In addition, lower glum length, upper glum length, caryopsis length and caryopsis width (CV= 9.14, 9.19, 10.3 and 10.38, respectively) also showed low variation in the studied genotypes.

Cluster analysis was performed to classify the genotypes based on morphological traits. The genotypes were divided into four groups which more or less corresponded to their subspecies identities (Fig. 1). On the other hand, the distribution of the genotypes in the clusters did not show any relationship with their geographical origin. All three genotypes of *B. danthoniae* subsp. *pseudodanthoniae* were allocated exclusively to group 1, whereas 6 out of 7 genotypes of var. *lanuginusos* and 2 genotypes (out of 72) of subsp. *danthoniae* were included in group 3. The genotypes of subsp. *danthoniae* were included into two groups; 14 (out of 72) genotypes of subsp. *danthoniae* had more similarities to subsp. *pseudodanthoniae* and were included in group 2, while the remaining 56 genotypes were included in group 4.

The results of factor analysis revealed that the first three factors encompass about 51% of total variation. The first factor with 28% of total variation comprised floret number per spikelet, lemma width, upper glume width, lemma length, Spikelet length and Awn length (Table 4). The second factor with 13% of total variation included the phenological traits including days to anthesis, days to maturity and days to flowering. Finally, the third factor with about 10% of total variation comprised awn number and tiller number.

Table 2. Results of analysis of variance for the quantitative traits tested in 82 *Bromus danthoniae* genotypes

Trait	Mean square		
	Genotype (df = 81)	Replication (df= 1)	Error (df = 81)
Length of longest internode	001.26**	0002.39	0.32
Spike length exclude awn	004.88**	0032.13	1.56
Spike width	001.35*	0001.94	0.42
Spikelet per spike	019.17**	0012.36	3.91
Spikelet length	000.17**	0000.005	0.042
Spikelet width	000.024**	0000.0018	0.016
Floret number per spikelet	004.41**	0000.27	1.48
Fertile floret number	006.30**	0001.25	2.02
Awn number	000.21**	0000.02	0.012
Awn length	000.11**	0000.00005	0.023
Lemma length	005.93**	00000.52	0.66
Lemma width	001.08**	0001.002	0.26
Caryopsis length	002.78**	0000.19	0.38
Caryopsis width	000.04**	0000.0004	0.018
Upper glume length	000.93**	0000.93	0.41
Upper glume width	000.83**	0000.17	0.11
Upper glume nerve number	001.60**	0000.90	0.56
Lower glume length	000.56**	0000.12	0.26
Lower glume width	000.29**	0000.10	0.074
Lower glume nerve number	000.77**	0000.01	0.23
Length of inflorescence stalk	012.79**	0005.61	2.64
Length of spikelet stalk	013.17**	0084.31	4.89
Tiller number	393.04**	0034.3	31.24
Plant height	039.12**	0004.44	17.39
Days to flowering	023.34**	2802.95	7.76
Days to anthesis	022.22**	2769.98	11.25
Days to maturity	010.36**	4241.19	5.56

* and ** Significant at P <0.05 and 0.01 levels, respectively

Table 3. Statistical data for the quantitative traits studied in 82 *Bromus danthoniae* genotypes

Trait	Mean	SE (±)	Minimum	Maximum	Variance	CV (%)
Length of longest internode (cm)	3.2	0.07	1.74	06.90	0.80	27.95
Spike length exclude awn (cm)	7.31	0.14	1.81	14.33	3.40	25.23
Spike width (cm)	2.54	0.07	0.95	07.57	0.89	37.15
Spikelet no. per spike	8.22	0.24	3.8	21.00	9.68	37.85
Spikelet length (cm)	2.05	0.03	1.37	03.49	0.11	15.98
Spikelet width (cm)	0.51	0.01	0.3	00.80	0.01	16.71
Floret number	9.58	0.13	5.69	14.67	2.93	17.85
Fertile floret number	6.9	0.16	1.5	12.50	4.15	29.50
Awn number	2.94	0.03	1.0	03.00	0.11	11.33
Awn length (cm)	1.79	0.02	1.07	02.55	0.07	14.25
Lemma length (mm)	11.95	0.11	5.92	15.73	1.94	11.65
Lemma width (mm)	4.1	0.06	2.3	06.42	0.52	17.56
Caryopsis length (mm)	8.79	0.07	4.82	10.86	0.82	10.30
Caryopsis width (mm)	1.7	0.01	1.21	02.36	0.03	10.38
Upper glume length (mm)	8.71	0.06	6.4	11.16	0.64	09.19
Upper glume width (mm)	4	0.05	2.28	05.55	0.38	15.43
Upper glume nerve no.	8.74	0.08	5.25	11.97	1.08	11.90
Lower glume length (mm)	7	0.05	5.07	08.57	0.41	09.14
Lower glume width (mm)	2.23	0.03	1.34	03.24	0.18	19.16
Lower glume nerve no.	4.36	0.06	3.0	06.75	0.50	16.25
Length of inflorescence stalk (cm)	7.91	0.22	3.34	15.96	7.71	35.11
Length of spikelet stalk (mm)	7.72	0.24	0.61	16.68	9.50	39.91
Tiller number	19.77	1.13	7.5	110.00	211.06	73.49
Plant height (cm)	21.73	0.41	10	40.00	28.11	24.41
Days to flowering	175.09	0.45	159	195.00	32.66	03.26
Days to anthesis	187.65	0.45	171	203.00	33.63	03.09
Days to maturity	203.13	0.45	193	211.00	33.93	02.87

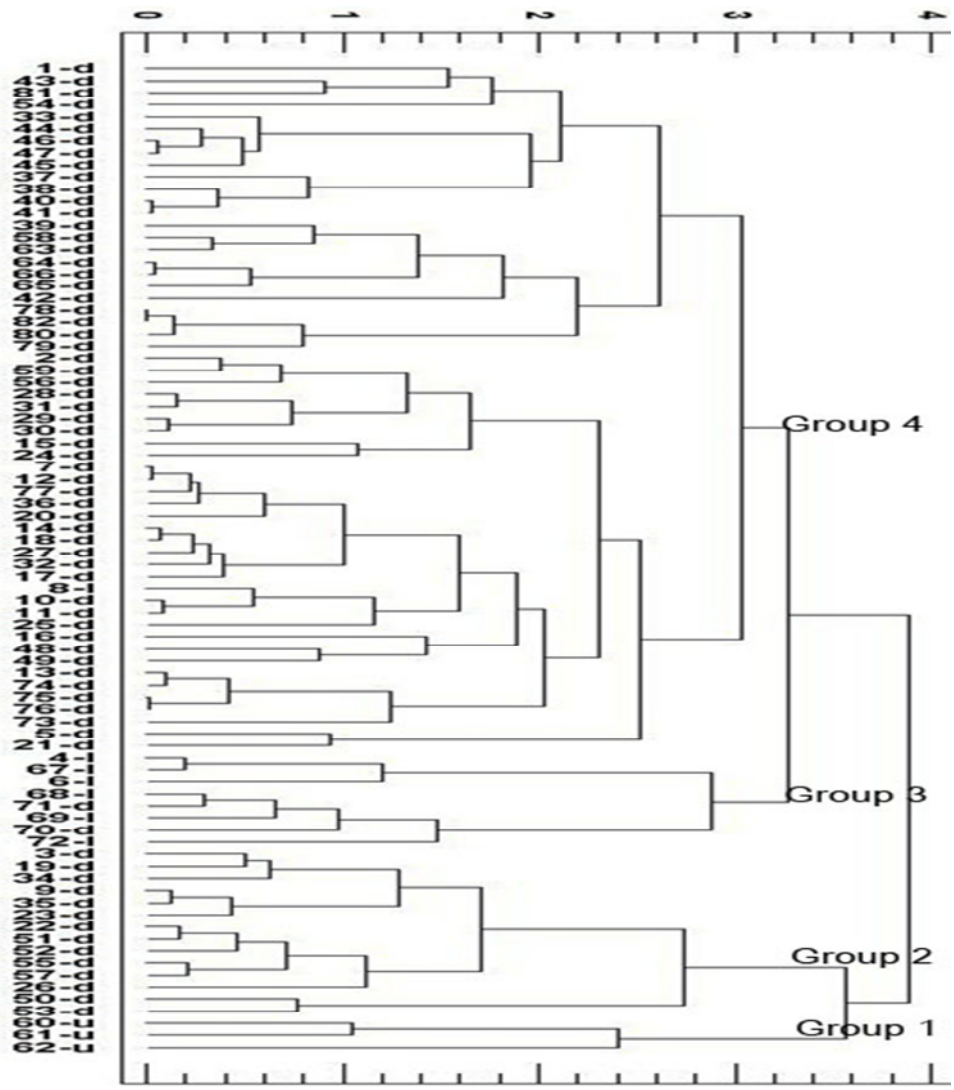

Fig. 1. Dendrogram generated based on morphological traits in *Bromus danthoniae* genotypes using Ward's method. For details of genotypes see Supplementary Table S1, letter d: subsp. *danthoniae*; I: var. *lanuginusos* Roshev; and u: subsp. *pseudodanthoniae* (Drobov) H. Scholz.

Results of correlation analysis showed that lemma length had strong positive correlations (P<0.01) with spike length excluding awn (r=0.32**), spikelet length (r=0.36**), floret number per spikelet (r=0.35**), fertile floret number (r=0.32**), awn length (r=0.55**), lemma width (r=0.72**), caryopsis length (r=0.84**), upper glume length (r=0.39**), upper glume width (r=0.535**), upper glume nerve number (r=0.34**), lower glume length (r=0.32**) and length of spikelet stalk (r=0.57**). In addition, lemma width was found to be significantly correlated (P<0.01) with the following traits: length of longest internode (r=0.353**), spike length exclude awn (r=0.36**), spikelet length (r=0.39**), floret number per spikelet (r=0.47**), fertile floret number (r=0.47**), awn length (r=0.44**), lemma length (r=0.72**), caryopsis length (r=0.58**), caryopsis width (r=0.31**), upper glume width (r=0.64**), upper glume nerve number (r=0.31**), lower glume width (r=0.47**), length of inflorescence stalk (r=0.39**) and length of spikelet stalk (r=0.51**).

Results of correlation analysis which explored the relationship between the phenological traits and the geographical coordinates of the origins of the collected genotypes showed that days to flowering, days to anthesis and days to maturity correlated highly and significantly with latitude (r = 0.52**, 0.57** and 0.51**, respectively), whereas they were negatively correlated with the longitude (r = 0.31**, 0.40** and 0.35** respectively). The relationships between these traits and altitude were found to be non-significant with the exception of low correlation coefficient obtained between day to maturity and altitude (r= 0.28*).

The results of cytological study showed that the somatic chromosome complement of the 25 tested genotypes can be classified into two distinct groups of diploid and tetraploid. As illustrated in Fig. 2a and b, *B. danthoniae* subsp. *danthoniae* and var. *lanuginusos* were diploid (2n=2*x*=14), whereas subspecies *pseudodanthoniae* was tetraploid (2n=4*x*=28) (Fig. 2c).

The results of ANOVA for anatomical traits showed significant differences (P<0.01) among the genotypes for the length of guard cell of stomata (stomatal length), epidermal cell length and the density of stomata (Table 5). A representative sample of upper leaf surface structure of the three taxonomic groups of the studied genotypes which included *B. danthoniae* subsp.

danthoniae, var. *lanuginusos* and subsp. *pseudodanthoniae* was illustrated in Fig. 3a, b and c, respectively. Orthogonal polynomials are commonly used in the analysis of variance to construct the orthogonal contrasts among equally spaced levels of a treatment factor. In the current study, the orthogonal contrasts made between diploid genotypes vs. tetraploid genotypes for anatomical traits showed that there was a significant difference ($P<0.01$) between the ploidy levels for both the stomatal length and density but not for the epidermal cell length. In addition, diploid genotypes significantly varied for the stomatal length and the epidermal cell length.

Table 4. Factor analysis result based on morphological traits in 82 *Bromus danthoniae* genotypes. The values indicate the correlation of the traits with each of the three factors

Trait	Factor		
	1st (28%)	2nd (13%)	3rd (10%)
Length of longest internode	0.61	-0.24	-0.47
Spike length exclude awn	0.64	-0.25	-0.08
Spike width	0.59	-0.25	-0.12
Spikelet per spike	0.23	-0.58	-0.48
Spikelet length	0.74	-0.14	-0.08
Spikelet width	0.64	-0.13	0.21
Floret number per spikelet	0.79	0.01	0.07
Fertile floret number	0.64	-0.01	0.3
Awn number	-0.09	0.51	0.66
Awn length	0.71	0.34	0.17
Lemma length	0.74	0.34	-0.02
Lemma width	0.76	0.06	0.12
Caryopsis length	0.55	0.25	-0.01
Caryopsis width	0.28	0.1	0.35
Upper glume length	0.47	0.52	-0.39
Upper glume width	0.76	0.27	0.18
Upper glume nerve number	0.36	0.5	-0.17
Lower glume length	0.52	0.25	-0.22
Lower glume width	0.56	-0.1	0.35
Lower glume nerve number	0.21	-0.41	0.13
Length of inflorescence stalk	0.53	-0.23	-0.21
Length of spikelet stalk	0.61	0.06	0.06
Tiller number	0.15	-0.37	-0.61
Plant height	0.2	-0.18	-0.32
Days to flowering	0.17	-0.74	0.31
Days to anthesis	0.16	-0.62	0.52
Days to maturity	0.17	-0.69	0.36

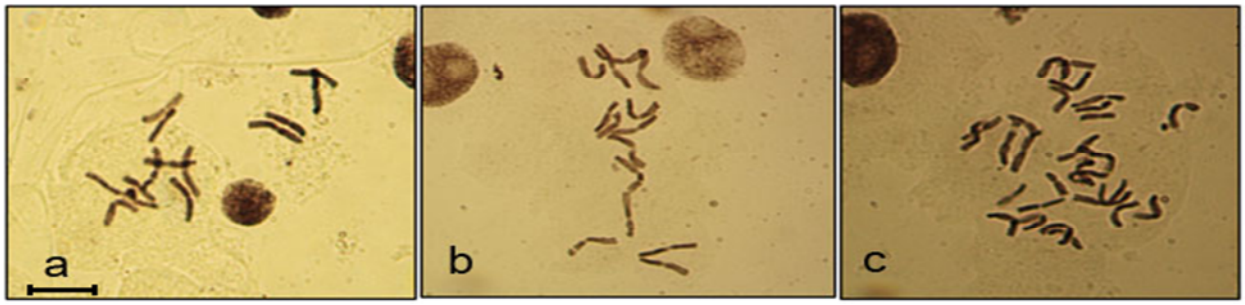

Fig. 2. Somatic chromosomes of *Bromus danthoniae* subsp. *danthoniae* (a), var. *lanuginusos* Roshev (b) and subsp. *pseudodanthoniae* (Drobov) H. Scholz (c) (scale bar: 20 µm)

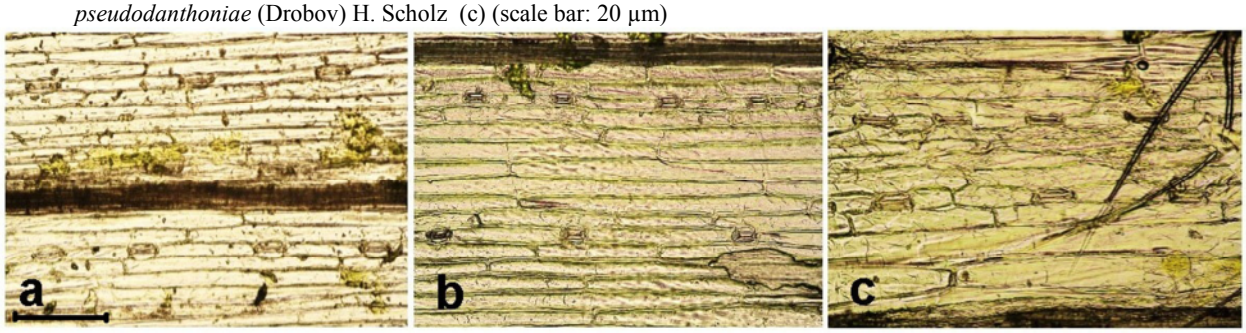

Fig. 3. Upper leaf surface structure of *Bromus danthoniae* subsp. *danthoniae* (a), var. *lanuginusos* Roshev. (b) and subsp. *pseudodanthoniae* (Drobov) H. Scholz (c) (scale bar: 200 µm)

Table 5. Results of analysis of variance for anatomical traits in three sub-taxa of *Bromus danthoniae* genotypes

Source of variation	df	Mean square		
		Stomatal length	Epidermal cell length	Stomatal density
Genotype	5	2050.22**	260072.03**	4.75**
Diploids vs. Tetraploids	1	5958.54**	7047.97ns	17.82**
Between diploids	3	1413.72**	287170.69**	1.19ns
Between tetraploids	1	51.41ns	431800.12**	2.38*
Residual	35	43.75	44516.09	0.34

ns Non-significant, * and ** significant at P <0.05 and 0.01, respectively

The evaluated values for Shannon index for qualitative traits indicated that there was a high genetic variation for stem cover and stem cover at inflorescence with high values of their Shannon index in *Bromus danthoniae* genotypes but genetic variation for lemma shape and lemma texture was very low in these genotypes.

Results of the current study indicated that there were high morphological variations among the genotypes which were independent of their geographical origin. These findings are in agreement with those of Aulicino and Arturi (2002) who observed high variation among populations of *B. catharticus* Vahl, for the reproductive traits. Likewise, high phenotypic variability and considerable plasticity for quantitative variables have been found especially for annual species of *Bromus* (Smith, 1981; Hufft and Zelikova, 2016).

Our results of cluster analysis are not consistent with those of Aulicino and Arturi (2008) who found a relationship between classification pattern of *B. catharticus* populations and the geographical origins. In their study, the sampled population groups corresponded to a gradient of temperature and humidity diminishing from the NE to the SW of Argentina. Highly stable morphological variables used in our cluster analysis, different species and ecological conditions could be forwarded to explain the discrepancies between our results and earlier publications.

In the current study, the clustering of the genotypes was almost in accordance with their subspecies status showing the usefulness of the studied highly heritable morphological traits in genetic diversity assessment of *B. danthoniae*. On the other hand, low variations of some of the traits such as lower glum length, upper glum length, caryopsis length and caryopsis width are in agreement with the findings of Oja and Paal (2004) who reported that the traits related to upper and lower glum lengths were not informative in three annual *Bromus* species.

The results of factor analysis indicated that the first factor is mostly associated with some of the spikelet attributes including lemma, glum and awn. These traits had been used as discriminators for taxonomical investigations to recognize *Bromus* species (Bor, 1970; Scholz, 1998) and the presence of three sub-taxa of *B. danthoniae* genotypes in this study could confirm this association. The second factor had the highest association with phonological traits. Finally, the third factor had the highest relationship with awn number and tiller number, which was related to sub-taxa characteristics in *B. danthoniae,* in the way that the genotypes belonging to subsp. *Pseudodanthoniae* were tetraploid and had the lower awn number and higher tiller number than other genotypes. Thus, it could be concluded that most of the variations among studied genotypes could be explained by the traits which were directly or indirectly related to their sub-taxa characteristics.

The results of correlation analysis showed strong and significant relationships between lemma length and lemma width with other inflorescent traits. In addition, these two traits were included in the first factor in factor analysis showing their strong relationships. The current study highlighted the potential use of either length or wide lemma singly or in combination as the most critical inflorescence components for within species discrimination. The length of Lemma has been suggested as the most practical variable in the systematic studies of *Bromus* spp. due to its great discriminative power and good conservation during specimen preparation (Aulicino and Arturi, 2008).

Despite the very low variation of phenological traits among studied genotypes, they had strong relationships with geographical coordinates of the origins of collected seeds. It is reasonable to postulate the existence of a biological base for phenological development whose maturation is essentially unaffected by the environmental factors in *B. danthoniae*. Hence, phenological traits can be considered as highly heritable attributes (Anderson et al., 2012). Many controversial observations have been reported regarding the relevance of environmental affected traits and latitude and longitude of the source of seeds. According to Johnson et al., (2010) the correlation coefficients between environmental variables like latitude and longitude and *Bromus carinatus* Hook. and Arm. variations were not significant while it was strongly significant for altitude. Otherwise, Erickson et al. (2004) found that phenotypic variation was associated with longitude in blue wild rye (*Elymus glaucus* Buckley).

To investigate intra-species differences of *B. danthoniae* genotypes, the chromosome number and ploidy level of these genotypes were checked. The ploidy level of genotypes belonging to *B. danthoniae* subsp. *pseudodanthoniae* was tetraploid (2n=4x=28) while the others were diploid (2n=2x=14). Ploidy level is a crucial factor in plant breeding because it can impact considerably on plant vigor, female and male fertility, cross fertility, and gene expression (Contreras

et al., 2007). This implies that polyploid plants may have desirable traits including enhanced vigor, thicker leaves, larger flowers, and higher persistence (Jones et al., 2007). Accordantly, in the present study, the genotypes belonging to tetraploid, *B. danthoniae* subsp. *pseudodanthoniae* had higher number of tillers, larger spikes and spikelets and thicker leaves than other sub-taxa (two diploids). These characteristics that result in larger vegetative organs are most plausible to forage and pasture crops (Arzani and Ashraf, 2016).

Our findings of larger guard cells of stomata but fewer density of stomata in tetraploid genotypes compared with diploid genotypes are consistent with those reported earlier for spontaneous *Arabidopsis* tetraploid ecotype (Monda et al., 2016) and *Acacia mearnsii* (de Wild) (Beck et al., 2003), who suggested the stomatal frequency and stomatal length as rapid indirect means to ascertain ploidy level. On the other hand, Nwokeocha (2015) found the slight variation of stomatal length among the accessions and could not reliably identify diploids from tetraploid in *Oryza punctata* Kotschy ex Steud. Furthermore, the significant difference among diploid genotypes for stomatal length was detected, which may be caused by environmental conditions. Our findings are consistent with the previous studies (Lomax et al., 2014) which indicated that the stomatal sizes and some of other cells in leaves are related to genome size and are also influenced by environmental conditions in *Allium species*. In addition, our results indicate that a comprehensive relationship between genome size and guard cell size cannot be ruled out, considering that larger guard cells of stomata and a fewer density of stomata in the leaves were found in tetraploid plants compared to the diploid plants. Therefore, to differentiate ploidy level in this species, both stomatal length and stomatal density should be considered together to be used as a reliable shortcut.

CONCLUSIONS

The high morphological and anatomical variations observed among the *B. danthoniae* genotypes reveal the evolutionary dynamics responsible for the distinct properties of the tested genotypes. These, in turn, explain that the habitat distribution of this species contributes to adapting harsh climates and stressful conditions, and enable us to improve this species in the plant breeding programs for enhancing pasture and grassland production. Finally, further research into the variability of this species from other distribution regions will improve our understanding of its phenotypic plasticity and evolutionary adaptation.

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تنوع مورفو-فنولوژی و تعدادکروموزوم در ژنوتیپ‌های ایرانی *Bromus danthoniae* Trin

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چکیده- گونه *Bromus danthoniae* Trin. گراس یکساله است که اساساً در شیب‌ها و دامنه‌های صخره‌ای خشک و مناطق استپی رشد می‌کند، توسط بسیاری از حیوانات چرا شده و به عنوان یک گیاه مرتعی کارا شناخته می‌شود. عدد کروموزومی و صفات مورفولوژی و آناتومی ۸۲ ژنوتیپ از این گونه (شامل سه زیر گونه) مورد بررسی قرار گرفت و تعداد ۲۷ صفت کمی و ۲۰ صفت کیفی اندازه‌گیری شد. نتایج تجزیه واریانس نشان داد ژنوتیپ‌های مورد مطالعه در همه صفات کمی آزمون شده تفاوت معنی دار داشته‌اند. بر مبنای نتایج حاصل از تجزیه کلاستر ژنوتیپ‌ها در چهار گروه قرار گرفتند که عمدتاً با گروه‌بندی بر اساس زیرگونه مطابقت داشت. همچنین نتایج حاصل از بررسی همبستگی‌ها نشان داد طول لما همبستگی مثبت و بالایی با صفات دیگر مانند طول سیخک (r=۰/۵۵)، عرض لما (r=۰/۷۲) و طول دانه (r=۰/۸۴) داشت. علاوه بر آن، عرض لما همبستگی بالا و معنی‌داری با سایر صفات مانند تعداد گل در سنبلچه (r=۰/۴۷) و طول دانه (r=۰/۵۸) داشت. نتایج تجزیه به عامل‌ها نشان داد سه عامل اول توانست در مجموع ۵۱٪ از تنوع کل را توجیه کند. بطور کل، صفات طول و عرض لما قابل اعتماد ترین صفات در مطالعات مورفولوژی این گونه بوده است. همچنین نتایج نشان داد *B. danthoniae* subsp. *danthoniae* و *B. danthoniae* var. *lanuginusos* Roshev. دیپلویید (۲n=۲x=۱۴)، در حالیکه *B. danthoniae* subsp. *pseudodanthoniae* (Drobov) H. Scholz تتراپلویید (۲n=۴x=۲۴) است. بر اساس نتایج حاصل از بررسی‌های آناتومی سطح برگ، ژنوتیپ تتراپلویید دارای اندازه روزنه بزرگتر و تراکم روزنه کمتر نسبت به ژنوتیپ‌های دیپلویید بود. بنابراین وجود ارتباط جامع بین اندازه ژنوم و اندازه سلول‌های نگهبان روزنه به عنوان معیاری برای تمایز سطوح پلوییدی متفاوت این گونه رد نخواهد شد. تنوع مورفولوژی بالا بین ژنوتیپ-های مطالعه شده، پراکندگی وسیع رشدی آن را توصیف کرده و ما را قادر می‌سازد که با استفاده از این گیاه در برنامه‌های اصلاحی گیاهان، تولیدات مرتعی را افزایش دهیم.