

TAXONOMIC APPLICATIONS OF SEED PROTEINS IN THE GENUS *BROMUS* L. (POACEAE)

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Received: 02.06.2008. Accepted for publication: 24.11.2008

Sheidai, M., Saeidi, S. & Atri, M. 2008 12 31: Taxonomic applications of seed proteins in the genus *Bromus* L. (*Poaceae*)-Iran. *J. Bot.* 14 (2): 126-131. Tehran.

Seed proteins were studied in 21 populations of 17 *Bromus* species and varieties growing in Iran. In total 83 protein bands were observed in all the species studied. The number of bands and their density varied in these species, indicating their genetic differences. Some specific bands were observed in most of the species indicating the presence of specific genes in these species as a result of species diversification. Such specific protein bands may be of use in the *Bromus* species identification. Different clustering and ordination methods showed separation of the species of sect. *Bromus* from the species of the sect. *Pnigma*, indicating the use of seed proteins in taxonomic delimitation of these two sections.

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Key words. *Bromus*, *Poaceae*, seed proteins, Iran.

کاربرد تاکسونومیکی پروتئین های بذر در جنس *Bromus* L.

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پروتئین های ذخیره ای بذر در ۲۱ جمعیت ۱۷ گونه و اریته *Bromus* ایران بررسی شد. بطور کلی تعداد ۸۳ باند پروتئینی مشاهده شد. گونه های مطالعه شده در تعداد و شدت باند تفاوت داشتند که بیانگر تفاوت های ژنتیکی آنهاست. تعدادی باند منحصر به فرد در اکثر گونه ها وجود داشتند که نشان دهنده وجود ژن های خاص در این گونه هاست که در طی گونه زایی بدست آمده اند. از این باندهای منحصر به فرد می توان در شناسایی و تفکیک گونه های *Bromus* استفاده کرد. روش های مختلف تجزیه خوشه ای و رسته بندی جدایی گونه های دو بخش *Bromus* و *Pnigma* را نشان داد.

INTRODUCTION

The genus *Bromus* L. (tribe *Bromeae*, *Poaceae*) is a well-defined natural group of grasses which comprises about 160 annual and perennial species (Acedo & Liams 2001), distributed all over the world with greatest variation in Europe and Asia (particularly South-West Asia) Stebbins 1981). The *Bromus* species are typically cool-season grasses, varying greatly in adaptation and use, including some important forage and range species, such as *B. inermis* Leyss., *B. anomalus* Rupr. ex E. Fourn., *B. pumpellianus* Scribn., *B. catharticus* Vahl, *B. mollis* L., and *B. rigidus* Roth (Carlson & Newell 1985). Basic chromosome number

is $x = 7$ and chromosome numbers vary from $2n = 14$ to $2n = 84$, most of the species being diploid ($2n = 14$) or tetraploid ($2n = 28$) (Federov 1969).

Bor (1970) in *Flora Iranica*, places the *Bromus* species in six sections. The present study reports seed protein analysis in 21 populations of 17 *Bromus* species and varieties belonging to the sections *Bromus* and *Pnigma* Dumort.

MATERIAL AND METHODS

Plant materials

Seed proteins were studied in 21 populations of 17 *Bromus* species and varieties growing in Iran (Table 1).

The species and varieties studied are: 1- *B. scoparius* L. var. *scoparius*, 2- *B. japonicus* Thunb. var. *japonicus*, 3- *B. japonicus* Roth. var. *velutinus* (Koch) Bornm., 4- *B. squarrosus* L. var. *squarrosus*, 5- *B. rechingeri* Melderis, 6- *B. lanceolatus* Roth. var. *lanceolatus*, 7- *B. briziformis* Fisch. et May., 8- *B. brachystachys* Hornung., 9- *B. secalinus* L., from the section *Bromus* and 10- *B. kopetdaghensis* Drobov., 11- *B. tomentellus* Boiss., 12- *B. variegatus* M. B., 13- *B. erectus* Hudson, 14- *B. cappadocicus* Boiss & Balansa, 15- *B. tomentosus* Trin., 16- *B. stenostachys* Boiss., and 17- *B. biebersteinii* Roemer & Schultes from the section *Pnigma* Dumort.. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU) and TARI.

SDS-PAGE Protein electrophoresis

Seed protein extraction and electrophoresis: Three hundred mg of seeds were collected randomly from different plants of each species or population and homogenized to obtain a fine powder. Proteins were extracted in a precooled mortar and pestle over ice with a 0.39 M Tris phosphate buffer (pH 8.3). The resulting mixture was centrifuged at 15000g for 10 min. The protein electrophoresis was carried out according to Sanchez-Yelamo et al. (1995), using 77mM Tris-HCl (pH 6.8), 4% sodium dodecyl sulphate (SDS), 10% 2-mercaptoethanol and 3% glycerol and vertical slab gels of 1 mm thickness which were electrophoresed at a constant rate of 30 mA for 8h. Coomassie Brilliant Blue G-250 was used for overnight gel staining followed by trichloroacetic acid as fixative. To estimate species/population similarity as indicated by protein electrophoresis patterns, bands having similar relative mobility (RM) were taken as homologue and Jaccard and simple matching indices were determined. Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). The resulting data matrix was used for cluster analysis using single linkage and UPGMA (unweighted paired group with arithmetic average) as well as principal coordinate (PCO) and principal components (PCA) analyses (Podani 2000, Sheidai et al. 2001).

RESULTS AND DISCUSSION

SDS-PAGE protein analysis has been used widely in biosystematic studies as seed storage proteins are less affected by environmental conditions, polyploidy level, etc. and are considered as good genetic markers for evolutionary studies (Chen et al. 1997). In total 83 protein bands were observed in all the species studied. The number of bands and their density varied in these species (Fig. 1), indicating their genetic differences.

Some specific protein bands were identified in the species studied. For example bands number 1 and 50 occurred only in *B. kopetdaghensis*, bands 2 and 25 occurred in *B. stenostachys*, band 23 and 41 were specific for *B. variegatus*, band 34 occurred only in *B. biebersteinii*, bands 51, 66, 77 and 80 occurred only in *B. secalinus*, band 56 occurred in *B. squarrosus*, band 65 in *B. briziformis*, band 68 in *B. brachystachys*, band 69 in *B. squarrosus* while bands 74 and 79 occurred in *B. lanceolatus* var. *lanceolatus*. The presence of specific bands indicates the occurrence of specific genes in the species studied due to species diversification. Such specific protein bands may be of use in the *Bromus* species identification.

Different clustering and ordination methods showed separation of the species of sect. *Bromus* from the species of the sect. *Pnigma* (Figs. 2 & 3), indicating the use of proteins in taxonomic delimitation of the species in these sections. Moreover populations of a single species were grouped together indicating the use of protein data in the species delimitation.

In general two major clusters are formed, each comprised of the species of one section. The first major cluster (including the species of sect. *Bromus*), is comprised of two sub-clusters. In the first sub-cluster, two varieties of *B. japonicus* var. *japonicus* and *B. japonicus* var. *velutinus* are placed close to each other due to their protein similarities. *B. rechingeri* shows similarity to these varieties and is placed close to them. *B. rechingeri* is a tetraploid species (Sheidai & Nourozi 2005) and we do not know if it is an allotetraploid species and whether *B. japonicus* genome is involved in its formation. The species of *B. japonicus* and *B. rechingeri* have been placed close to each other in Flora Iranica (Bor 1970).

Two species of *B. briziformis* and *B. squarrosus* show more similarity and are joined together. Smith (1972) in his serological studies also showed similarities between these two species. In the second sub-cluster, two species of *B. brachystachys* and *B. scoparius* L. var. *scoparius* show similarity supporting their morphological relationship (Smith and Sales 1993). *B. lanceolatus* var. *lanceolatus* and *B. secalinus* join each other with some distance. *B. secalinus* then shows more similarity to *B. brachystachys* and *B. scoparius* which is also suggested by serological studies (Smith 1972).

The second major cluster (including the species of sect. *Pnigma*) shows presence of 3 sub-clusters. In the first sub-cluster, two populations of *B. erectus* show similarity and are placed together. The species of *B. tomentellus* and *B. cappadocicus* show similarity and are placed close to each other, supporting their

Table 1. *Bromus* species their localities and collectors.

Species	Locality and collectors
<i>B. biebersteinii</i> Roemer & Schultes	Seiyahbisheh, Kala village, 1892 m, Saeidi 2003
<i>B. brachystachys</i> Hornung.	Mazandaran, Noor, Nouroozi & Amini 2005
<i>B. briziformis</i> Fisch. et May.	Astara, Heiran, Nouroozi & Parivand 2005
<i>B. cappadocicus</i> Boiss & Balansa	Hamedan, Aghbolagh, 1983 m, Saeidi 2003
<i>B. cappadocicus</i> Boiss & Balansa	Kojoor, 1620 m, Saeidi 2003
<i>B. erectus</i> Hudson	Gachsar, 2500 m, Saeidi 2003
<i>B. erectus</i> Hudson	Reineh towards Polour, 2292 m, Saeidi 2003
<i>B. japonicus</i> Thumb.var. <i>japonicus</i>	Karaj, Nouroozi & Parivand 2005,
<i>B. kopetdaghensis</i> Drobov	Emamzadeh Hashem, 2645 m, Saeidi 2003
<i>B. lanceolatus</i> Roth. var. <i>lanceolatus</i>	Kojoor, Hasanabad, Nouroozi & Parivand 2005
<i>B. rechingeri</i> Melderis	Tehran, Kan road, Soleghan, Nouroozi & Parivand 2005
<i>B. scoparius</i> L.var. <i>scoparius</i>	Astara, Heiran, Nouroozi & Parivand 2005
<i>B. squarrosus</i> L. var. <i>squarrosus</i>	Meshkinshahr, Nouroozi & Parivand 2005
<i>B. stenostachys</i> Boiss.	Gachsar, Nessa, 2087 m, Saeidi 2003
<i>B. tomentellus</i> Boiss	Nahavand, Ardooshan mountain, 1844 m, Saeidi 2003
<i>B. tomentellus</i> Boiss.	Abali, 2300 m, Saeidi 2003
<i>B. tomentellus</i> Boiss.	Hezarcham, Chaloos road, 1466 m, Saeidi 2003
<i>B. tomentellus</i> Boiss.	Larijan, Abegarm, 2086 m, Saeidi 2003
<i>B. tomentosus</i> Trin.	Chaloos, Hezarcham, 1466 m, Saeidi 2003
<i>B. tomentosus</i> Trin.	Seiyahbisheh, 2030 m, Saeidi 2003
<i>B. variegatus</i> M. B.	Baladeh towards Alamdeh, 2900 m, Saeidi 2003

taxonomic treatment in Flora Iranica (Bor 1970). The other species of the sect. *Pnigma* form the third sub-cluster.

PCA analysis of protein data showed that the first 6 factors comprise about 70% of total variance. In the first factor with about 30% of total variance, bands number 3,4, 18, 32, 40, 42, 43, 45 and 49 possessed the highest positive correlation (>0.70) while bands number 52, 53, 54, 55, 58, 61, 76, 82 and 83 possessed the highest negative correlation (<0.7). In the second factor with about 11% of total variance, bands number 10, 15, 29, 30 and 44, showed the highest positive or negative correlations. Therefore these protein bands are the most variable bands among the species studied. The bands of the first factor separate the species of two sections from each other while the bands of the second factor mainly separates the species of *B. tomentellus* and *B. cappadocicus* from the other species of sect. *Pnigma* (Fig. 4).

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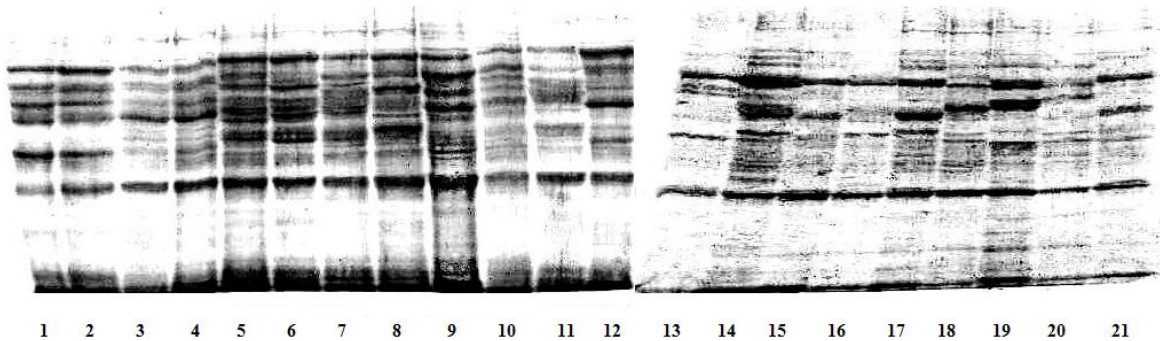


Fig. 1. SDS-PAGE protein profile of *Bromus* species studied.

Species code: 1 & 2 Abali and Nahavand populations of *B. tomentellus*, 3 & 4 = Hamedan and Kojoor populations of *B. cappadocicus*, 5 = *B. biebersteinii*, 6 & 7 = Larijan and Hezarcham populations of *B. tomentosus*, 8 = *B. stenostachyus*, 9 = *B. variegatus*, 10 & 11 = Alamdeh and Reineh populations of *B. erectus*, 12 = *B. kopetdaghensis*, 13- *B. japonicus* var. *japonicus*, 14- *B. briziformis*, 15- *B. secalinus*, 16- *B. scoparius* var. *scoparius*, 17- *B. squarrosus*, 18- *B. brachystachys*, 19- *B. japonicus* var. *velutinus*, 20- *B. lanceolatus* var. *lanceolatus*, and 21- *B. rechingeri*.

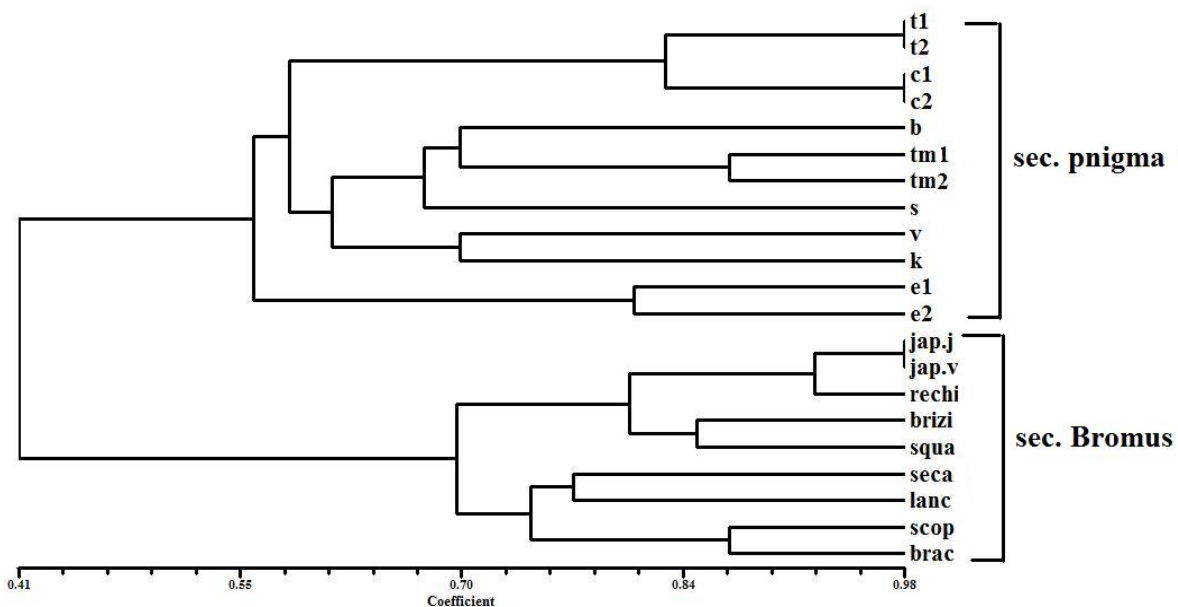


Fig. 2. UPGMA dendrogram of *Bromus* species studied.

Species abbreviations: t1 & t2 = Abali and Nahavand populations of *B. tomentellus*, c1 & c2 = Hamedan and Kojoor populations of *B. cappadocicus*, b = *B. biebersteinii*, tm1 & tm2 = Larijan and Hezarcham populations of *B. tomentosus*, s = *B. stenostachys*, v = *B. variegatus*, k = *B. kopetdaghensis*, e1 & e2 = Alamdeh and Reineh populations of *B. erectus*, , jap.j = *B. japonicus* var. *japonicus*, jap.v = *B. japonicus* var. *velutinus*, rechi = *B. rechingeri*, brizi = *B. briziformis*, squa = *B. squarrosus*, seca = *B. secalinus*, lanc = *B. lanceolatus* var. *lanceolatus*, scop = *B. scoparius* var. *scoparius*, brac = *B. brachystachys*.

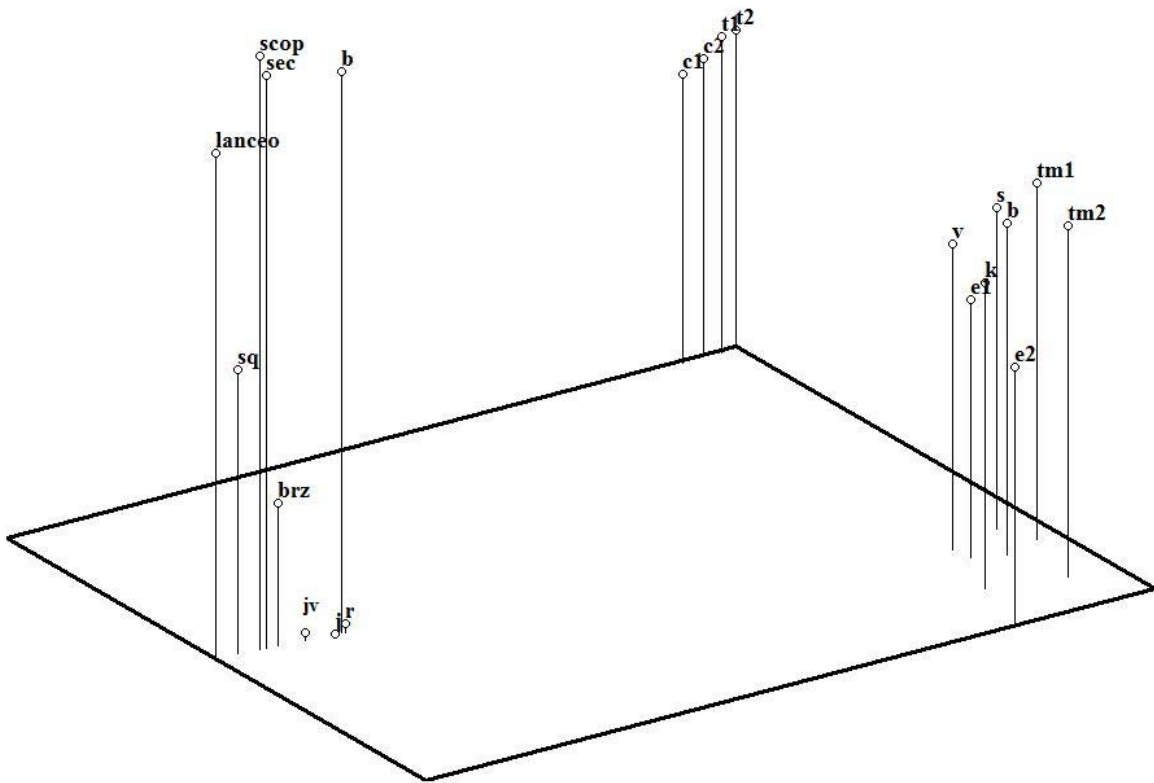


Fig. 3. PCO plot of *Bromus* species (species abbreviations as in Fig. 2).

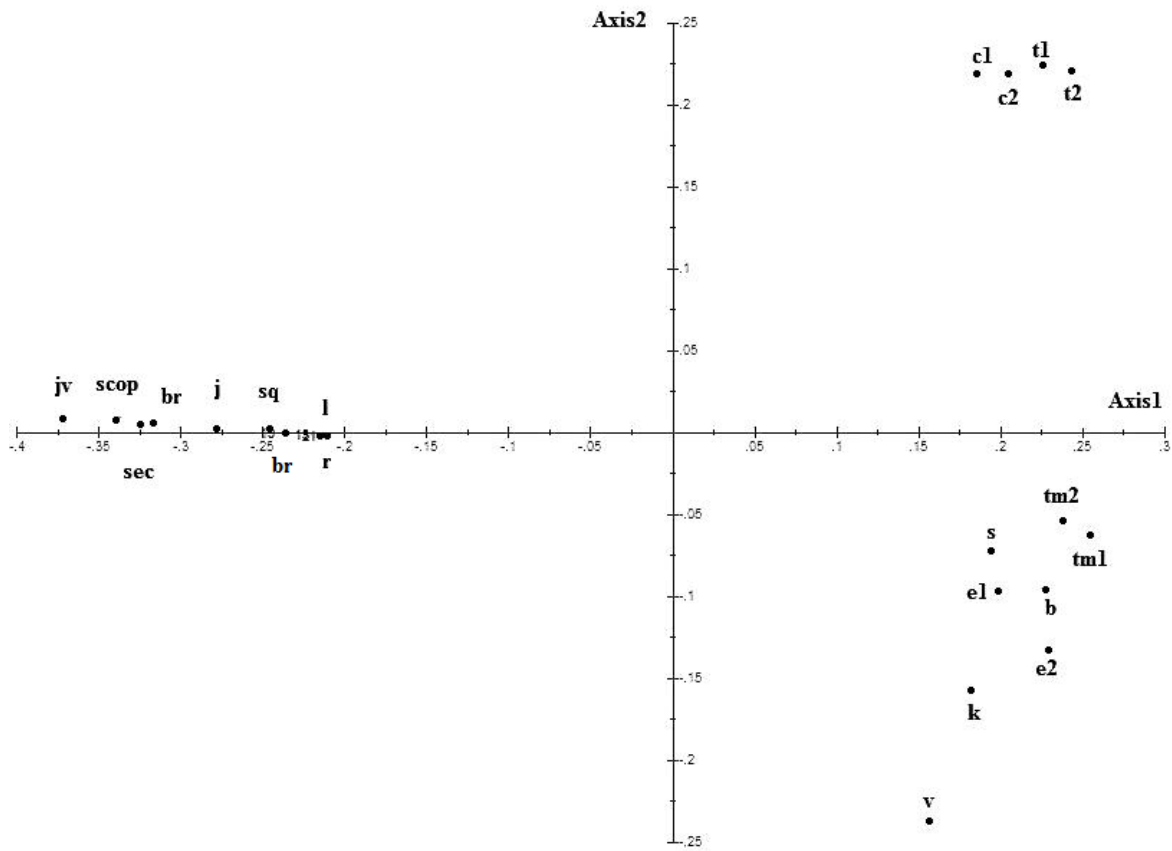


Fig. 4. PCA plot of *Bromus* species .

Species abbreviations: t1 & t2 = Abali and Nahavand populations of *B. tomentellus*, c1 & c2 = Hamedan and Kojoor populations of *B. cappadocicus*, b = *B. biebersteinii*, tm1 & tm2 = Larijan and Hezarcham populations of *B. tomentosus*, s = *B. stenostachys*, v = *B. variegatus*, k = *B. kopetdaghensis*, e1 & e2 = Alamdeh and Reineh populations of *B. erectus*, j = *B. japonicus* var. *japonicus*, jv = *B. japonicus* var. *velotinus*, rechi = *B. rechingeri*, br = *B. briziformis*, sq = *B. squarrosus*, sec = *B. secalinus*, l = *B. lanceolatus* var. *lanceolatus*, scop = *B. scoparius* var. *scoparius*, brac = *B. brachystachys*, r = *B. rechingeri*.