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Regional variation in Argentinean populations of *Bromus catharticus* (Poaceae) as measured by morphological divergence associated with environmental conditions

by

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Abstract

Aulicino, M.B. & Arturi, M.J. 2008. Regional variation in Argentinean populations of Bromus catharticus (Poaceae) as measured by morphological divergence associated with environmental conditions. *Anales Jard. Bot. Madrid* 65(1): 135-147.

Thirty-one populations of *Bromus catharticus* Vahl., collected from the Pampean Dominion (Argentina), were classified using twenty four highly heritable traits by numerical taxonomic methods. After implementing a stepwise discriminant analysis, 18 traits were chosen as classificatory variables. Eight population groups were classified in two main clusters. Different morphotypes, primarily associated with panicle architecture and micro floral traits, were found. The patterns in the morphological variation seem to correspond to a gradient of humidity and temperature that diminishes from the NE to the SW. This pattern of classification reflects the geographical origin for most of the sampled populations, although there was some noise. Our results fit the patchy variation model, where populations are genetically selected for macro and micro environmental conditions.

Keywords: *Bromus catharticus*, genetic variation, morphotypes, numerical taxonomy, phenotypic variation, quantitative traits, rescue grass.

Resumen

Aulicino, M.B. & Arturi, M.J. 2008. Modelo de variación regional en germoplasma de Bromus catharticus (Poaceae) en Argentina asociado con las condiciones ambientales. *Anales Jard. Bot. Madrid* 65(1): 135-147 (en inglés).

Treinta y una poblaciones de *Bromus catharticus* Vahl., recolectadas en el dominio Pampeano (Argentina), fueron clasificadas utilizando 24 caracteres altamente heredables por métodos de taxonomía numérica. Tras implementar el análisis discriminante del paso a paso (Stepwise), 18 variables fueron seleccionadas como variables clasificatorias. Ocho grupos de poblaciones fueron clasificadas en dos clusters principales. Diferentes morfotipos, principalmente asociados a la arquitectura de las panojas y a variables microflorales, fueron encontrados. El patrón de variación morfológico parece responder a un gradiente de humedad y temperatura que disminuye desde el NE al SW. Además, dicho patrón de clasificación refleja un origen geográfico para la mayoría de las poblaciones, aunque hubo algo de ruido. Nuestros resultados se ajustan a un modelo de variación en parches, donde las poblaciones están genéticamente seleccionadas por condiciones micro y macro ambientales.

Palabras clave: *Bromus catharticus*, variación genética, morfotipos, taxonomía numérica, variación fenotípica, variables cuantitativas, cebadilla criolla.

Introduction

Bromus L. is a taxonomically difficult genus with a wide distribution (> 150 species) over temperate regions of both hemispheres (Clayton & Renvoize, 1986; Saarela & al., 2007). *Bromus* sect. *Ceratochloa* (P. Beauv.) Griseb. contains an extensive polyploid complex based on $x = 7$: *B. carinatus* Hook & Arn octoploid complex and *B. catharticus* hexaploid complex (Stebbins & Tobgy, 1944; Armstrong, 1981; Stebbins, 1981). In South America Smith (1970) recognized seven species in the *B. catharticus* complex: *B. catharticus* Vahl., *B. brevis* Nees ex Steud., *B. bonariensis* Parodi &

J.A. Cámara, *B. coloratus* Steud., *B. parodii* Covas & Itria, *B. stamineus* E. Desv., and *B. valdivianus* Phil. Gutiérrez & Pensiero (1998) also included in *Bromus* sect. *Ceratochloa, B. lithobius* Trin., *B. mango* E. Desv., *B. tunicatus* Phil., and *B. cebadilla* Steud. (taxonomic synonyms of *B. stamineus* and *B. valdivianus*). Intraespecific variability was also cited (Gutiérrez & Pensiero, 1998). Planchuelo (1991) suggested that *B. bonariensis*, *B. brevis,* and *B. catharticus* must be considered conspecific. Massa & al. (2004) recognized two subspecies: *B catharticus* ssp. c*atharticus* and *B. catharticus* ssp. *stamineus* (E. Desv.) A.N. Massa. In a previous paper, Peterson & Planchuelo (1998) identified two varieties within the *B. catharticus* complex: *B. catharticus* var. *catharticus* and *B. catharticus* var. *rupestris* (Speg.) Planchuelo & P.M. Peterson.

In Argentina, *B catharticus* (syn. *B. willdenowii* Kunth, *B. unioloides* Kunth) 'rescue grass' or 'prairie grass' is widely distributed in the Pampas (Burkart, 1969). It grows spontaneously in natural and disturbed areas. This region offers a large diversity of plant habitats due to heterogeneity of landscapes, soils and climates (Cappannini, 1968; Tricart, 1973); however, agricultural exploitation is reducing natural areas and the environmental quality of habitats. Due to this process, drastic genetic erosion might be occurring in this region in natural populations of plants (Richards, 1986).

Bromus catharticus is a facultative autogamous species where self-fertilization is more common than intercrossing (Ragonese & Marcó, 1941; Pérez López, 1975). Previous studies on prairie grass pointed out high phenotypic variability between populations, plastic responses to differing habitats, and low heritability for vegetative and reproductive traits (Pahlen, 1986; Garcia & Arturi, 1992; Szpiniak & al., 1995; Wolff & al., 1996; Pistorale & al., 1999; Aulicino & Arturi, 2002). Analysis of molecular markers confirmed low levels of genetic variability (Puecher & al., 2001; Massa & al., 2004). Low levels of variability could be explained by the autogamous reproductive system and by increased levels of habitat disturbance (Fischer, 2004). Inbreeding obviously restricts gene flow but should also increase genetic differentiation among populations (Loveless & Hamrick, 1984).

In systematics, the choice of characters is often problematic (Dunn & Everitt, 1982). It becomes more difficult when herbarium material is evaluated or when a repetitive experimental design in different environments is used. As Anderson (1989) pointed out, morphometric studies of plants, grown in a uniform environment, do not demonstrate a clear pattern of divergence among taxa when plastic variables are used. One of the most important steps is to decide which are the most suitable traits to determine the genetic distance which reflects the degree of relationship between populations (Arunachalam, 1981). The use of high heritable and stable traits has demonstrated to be useful for discrimination of taxa (Sánchez & al., 1993). In a previous study, we assessed the constancy and heritability ratios of 39 vegetative and reproductive characters of *B. catharticus* across different Argentinean populations (Aulicino & Arturi, 2002). As a consequence of this, we selected as descriptors the quantitative variables with higher heritability to classify the populations.

Those surveyed *B. catharticus* populations were also included in this paper to further investigate the relationships between phenotypic variation and ecological factors. This may help conserve and protect the native germplasm simply by adding new populations to the existing collections that are currently being conserved in the germplasm bank at the Instituto Fitotécnico de Santa Catalina, Universidad Nacional de La Plata, Buenos Aires. A "core collection" will be developed and will facilitate future use of the germplasm (Marshall & Brown, 1975; Brown, 1989).

The aims of our research were: a) to identify divergent groups of *B. catharticus* populations using highly heritable traits, and b) to associate the pattern of classification with the collecting site and environmental factors.

Materials and methods

Sampling

Samples from 31 populations of *B. catharticus* were collected in 30 locations of the Province of Buenos Aires, distributed in four different geographic regions: Ondulating Pampa, Sandy Pampa, Interhill Pampa, and Depressed Pampa. Original information describing the material sampled and places of collection is presented in Table 1. The sampling was planned to cover geomorphological and climatic differences.

Fifty mature reproductive tillers (belonging to different individuals) were collected in different locations, from an ecological homogeneous area. Ten caryopses per panicle were taken out to make a pool per each population. Seed pools were used to plant the trials.

Design of the experiment

Two trials were conducted during two consecutive years, using a randomized block design with four replications. Four rows 2.25 m long, spaced 0.40 m

Table 1. Collection details of specimens from 31 natural populations of *Bromus catharticus* and climatic variables of each location.

Population	Identification	Latitude South	Longitude West	Regions	Average maximum temperature (ºC)	Average minimum temperature (ºC)	Absolute maximum temperature (ºC)	Absolute minimum temperature (ºC)	Average temperature (ºC)	Average precipitation (mm)	Relative humidity (%)
San Vicente	SVICE	35º 01′	58º 25′	Ondulating Pampas	22.5	11.7	39.0	– 4	17.1	1003	72
Cañuelas	CAÑUE	35º 03′	58º 46′	Depressed Pampas	22.4	11.6	38.9	– 4	17.0	1003	72
Gral. Las Heras	LHERA	34º 47′	58º 51′	Ondulating Pampas	22.0	11.4	39.0	– 4	16.7	1110	72
Gral. Rodríguez	GRODR	34º 40′	58º 57′	Ondulating Pampas	22.2	11.4	39.5	– 4	16.8	1181	73
Pilar	PILAR	34º 28′	58º 55′	Ondulating Pampas	22.3	11.5	40.5	– 4	16.9	1241	75
Belén de Escobar	ESCOB	34º 21′	58º 48′	Ondulating Pampas	22.8	11.3	39.3	– 5.6	17.1	1111	76
Balcarce	BALCA	37º 52′	58º 15′	Interhill Pampas	19.7	8.2	38.0	– 4	13.9	932	79
Mercedes	MERCE	34º 40′	59º 26′	Ondulating Pampas	22.3	11.7	40.5	4	14.2	1006	72
Chivilcoy	CHIVI	34º 48′	59º 42′	Depressed Pampas	22.35	10.9	41.2	3	15.8	1013	73.5
Bragado	BRAGA	35º 07′	60º 30′	Sandy Pampas	22.5	10.5	42.3	2	16.3	1019	74.5
Carlos Casares	CCASC	35º 37′	61º 22′	Sandy Pampas	22.4	9.9	43.1	2	16.1	993	75
Carlos Casares b	CCASB	35º 37′	61º 22′	Sandy Pampas	22.4	9.9	43.1	2	16.1	993	75
Pehuajó	PEHUA	35º 48′	61º 54′	Sandy Pampas	22.2	9.5	43.4	2	15.0	968	76
Trenque Lauquen	TLAUQ	35º 58′	62º 44′	Sandy Pampas	22.8	9.9	43.7	2	16.4	955	68
Girondo	GIRON	35º 59′	61º 37′	Sandy Pampas	22.2	9.5	42.9	2	17.0	954	75
Bolivar	BOLIV	36º 14′	61º 07′	Sandy Pampas	22.2	9.5	42.4	2	15.8	941	74
Olavarría	OLAVA	36º 32′	60º 54′	Interhill Pampas	21	7.5	40.1	1	15.8	930	72
Azul	AZUL	36º 47′	59º 51′	Interhill Pampas	21	7.9	40.6	1	14.4	1004	78
Tapalqué	TAPAL	36º 21′	60º 01′	Depressed Pampas	21.6	8.5	40.6	2	16.4	968	76
Llavallol	LLAVA	34º 48′	58º 26′	Ondulating Pampas	22.3	10.6	41.2	– 5.1	16.4	938	73
Lezama	LEZAM	35º 52′	57º 54′	Depressed Pampas	21.3	9.0	39.7	– 7.4	15.2	891	81
Maipú	MAIPU	37º 06′	57º 50′	Depressed Pampas	21	7.0	39.6	– 7.7	14.0	856	66
Mar del Plata	MARDE	38º 00′	57º 33′	Interhill Pampas	18.7	10.6	38.6	– 3.2	14.6	1011	77
Magdalena	MAGDA	35º 05′	57º 31′	Ondulating Pampas	21.1	10.9	37.7	– 4.4	16.0	1000	77
Pipinas	PIPIN	35º 32′	57º 20′	Ondulating Pampas	23.9	11.1	42.6	– 7.3	17.5	860	68
Castellí	CASTE	36º 06′	57º 48′	Depressed Pampas	21.3	9.0	39.7	– 7.4	15.2	891	81
San Clemente	SCLEM	36º 22′	56º 24′	Depressed Pampas	19.2	11.8	45.6	– 6.6	15.5	865	85
Henderson	HENDER	36º 18′	61º 42′	Sandy Pampas	22.2	9.0	42.5	2	17.0	939	43
Las Flores	LFLOR	36º 01′	59º 06′	Depressed Pampas	21.5	8.1	38.7	– 7.9	14.8	959	76
Parque Pereyra	PEREY	34º 50′	58º 08′	Ondulating Pampas	21.3	10.7	37	– 4.5	16.0	1051	79
Punta Lara	PLARA	34º 55′	57º 57′	Ondulating Pampas	20.9	11.5	37.4	– 4.1	16.2	885	80

apart were the experimental units. Two hundred and fifty six seeds separated by 0.15 m were planted per plot (64 seeds per row). The trial were located in Llavallol, Buenos Aires (34°47'S 58°27'W), on an Argiudoll typical soil. Ten reproductive and completely developed but immature tillers were collected to measure floral attributes. Subsequently, they were dried and mounted on herbarium sheets. As consequence, vegetative and reproductive traits were measured over 40 dried tillers in each population and environment (number of specimens = 80).

Environmental factors

The selected sampling sites cover a gradient of humidity and temperature that diminishes from the northeast to the southwest.

The geomorphological areas have particular edaphic conditions. The Ondulating Pampa, a steep region that presents numerous ridges, is located in the northern portion of the province and has loamy well-structured soils probably due to higher humidity in the atmosphere. Sandy Pampa region has weakly structured sandy soils, probably due to less precipitation particularly in the western areas. Interhill Pampas region has tablelands with loess. The Depressed Pampa is a heterogeneous area that has a large diversity of habitats: depressions, plains with microsites of basins with saline and alkaline soils, riversides (Salado river), old packsaddles of marine deposits and coastal sandy strips (Cappannini, 1968; Tricart, 1973).

Geomorphological characteristics of the sampling sites were extracted from the soils chart of the Buenos Aires Province (Mapas de suelos de la Provincia de Buenos Aires, 1989). Climatic variables were taken from statistical charts (Estadísticas meteorológicas de la década 1981-1990 del Servicio Meteorológico Nacional, 1996) from meteorological stations nearest to the sampled localities (Ten years average) [see Table 1].

Abbreviations

DA - Discriminant analysis; CA - Cluster analysis; BSS - Between cluster sums of squares; PCA - Principal component analysis; KMO - Kaiser-Mayer-Olkin test.

Morphological traits and selection of characters

Floral attributes were estimated by averaging two glumes and lemmas per panicle. Number of florets per spikelet (NFS) and length of spikelets (LS) were obtained by averaging the measurements of four spikelets per panicle. Panicle attributes were estimated by averaging two primary branches per each panicle node. Distance between spikelets of the branches at the first node (DSFN) and distance between spikelets of the branches at the second node (DSSN) were estimated as a ratio between the length of the portion with flowers (of the branches at the first or second node) and the number of spikelets on this portion. The penultimate leaf is the uppermost one. Morphological traits, their units, and abbreviations are shown in Table 2.

Twenty-six variables with heritability over 20% were chosen. Heritabilities were estimated applying a genetic model using the univariate method described in Aulicino & Arturi (2002).

Given that some traits contained redundant information or were not good to discriminate populations, a stepwise discriminant analysis (DA) was used for character selection. A criterion based on the squared partial correlation was applied using the SAS PROC STEPDISC procedure of the SAS program (SAS Institute Inc., Cary, USA). All variables with p value less or equal to 0.15 were selected (Costanza & Afifi, 1979). The adequacy of sampling was estimated through the Kaiser-Meyer-Olkin (KMO) test, which was performed with the PROC FACTOR ANALYSIS of the SAS program.

Statistical analyses

We used population means of the selected variables to classify populations. The analysis began with a priori grouping of population using cluster analysis (Legendre & Legendre, 1998). The operational taxonomic units (OTUS) were the 31 populations. Since the characters were measured in different units, the data were standardized to zero for means and standard deviation equal to 1. After standardization, a distance matrix based on average taxonomic distance was computed (Sneath & Sokal, 1973). A comparison of distance matrices was done through a correlation Mantel test between matrices of the two years (Mantel, 1967), using NTSYS-pc 2.0 program (Rohlf, 1998).

Ward's minimum variance clustering method was used to classify populations a *priori*. To determine the exact number of clusters a pseudo t^2 statistic was displayed and then a plot of the pseudo t^2 statistic against the number of clusters was built (Khattree & Naik, 2000). The between cluster sum of squares (BSS) was used to measure the magnitude of the dissimilarity between clusters. An analysis of variance and a Tukey Test (using a significance level of $p < 0.05$) were performed to prove significant differences among the obtained population groups.

The unweighted pair-group and arithmetic average clustering method (UPGMA) and a principal component analysis (PCA) were used to corroborate the re-

sults of the cluster analysis based on Ward's minimum variance and to reveal the way in which individual descriptors contributed to the observed group structure. Euclidean distances from standardized descriptors were used to construct a dendrogram based on the average-linkage UPGMA. The cophenetic correlation coefficient (CCC) was used to determine the goodness of fit of the dendrogram (Sneath & Sokal, 1973) using the SAS PROC CLUSTER option. PCA was performed using a dissimilarity matrix based on the Pearson product-moment correlation coefficient (Sneath & Sokal, 1973). Those variables with a correlation coefficient higher than 0.65 were used to discriminate among populations (Sneath & Sokal, 1973), using the NTSYS-pc 2.0 program (Rohlf, 1998). Frontier's broken stick criterion allowed to evaluate the significance of the differences between the main PCA axes (Legendre & Legendre, 1998).

Environmental analysis

A multivariate linear regression analysis was conducted to determine the effect of the climate variables on the pattern of classification using SAS PROC REG program (Khattree & Naik, 2000). The projection scores of populations on the first three principal components (PCA1, PCA2 and PCA3) were used as response (dependent) variables, while environmental variables were used as predictor (independent) variables. The coefficient of determination R^2 and the F statistic test were taken as indices to measure the adequacy of the fitted model.

Results

Eighteen out of twenty-six morphological traits were chosen using the Partial Root Squares and F' values obtained by the stepwise procedure (Table 3) [Khattree & Naik, 2000]: upper glume width (FGW), average number of flowers per spikelets (NFS), lemma length (LL), lower glume width (SGW), flag leaf length (FLL), lemmatal awn length (LAL), average number of primary branches at the first and second panicle nodes (NBFSN), upper glume length (FGL), flag leaf sheath length (FSL), lemma width (LW),

Table 2. Morphological heritable traits (h^2 > 20%) measured on vegetative and reproductive organs of *Bromus catharticus*: names and abbreviations.

N.º	Traits	Abbreviation
	Morphological vegetative	
1	Flag Leaf Length, in cm	FLL
2	Flag leaf Width, in cm	FLW
3	Flag leaf sheath length, in cm	FSL
4	Penultimate leaf length, in cm	PLL
5	Penultimate leaf sheath length, in cm	PSL
	Morphological reproductive	
6	Number of nodes per panicle	NNP
7	Total number of primary branches per panicle	NPB
8	Total number of spikelets per panicle	NSP
9	Average number of florets per spikelet	NFS
10	Average Length of the spikelets, in cm	LS
11	Average number of primary branches at the first and second panicle nodes	NBFSN
12	Average number of primary branches at third and fourth panicle nodes	NBTFN
13	Average number of spikelets at the branches of the first and second panicle nodes	NSFSN
14	Average number of spikelets at the branches of the third and fourth panicle nodes	NSTFN
15	Distance between spikelets of the branches at the first node, in cm	DSFN
16	Distance between spikelets of the branches at the second node, in cm	DSSN
17	Upper glumes length, in cm	FGL
18	Upper glumes width, in cm	FGW
19	Number of nerves in the upper glumes	NNFG
20	Lower glumes length, in cm	SGL
21	Lower glumes width, in cm	SGW
22	Number of nerves in the lower glumes, in cm	NNSG
23	Lemma length, in cm	LL
24	Lemma width, in cm	LW
25	Number on nerves in lemma	NNL
26	Lemmatal awn length, in cm	LAL

Table 3. Stepwise selection summary of statistic parameters for the 18 selected traits of *Bromus catharticus*. Abbreviations as in Table 2.

Step number	Selected traits	Partial R square	F value	*p* value
1	FGW	0.25	2.44	0.0001
2	NFS	0.28	2.75	0.0001
3	LL	0.27	0.62	0.0001
4	SGW	0.27	0.53	0.0001
5	FLL	0.26	2.62	0.0001
6	LAL	0.29	2.47	0.0001
7	NBFSN	0.26	2.92	0.0001
8	FGL	0.25	2.44	0.0001
9	FSL	0.23	2.38	0.0002
10	LW	0.21	2.06	0.0018
11	NNP	0.22	1.79	0.0098
12	PLL	0.22	1.90	0.0050
13	SGL	0.22	1.94	0.0039
14	NNSG	0.22	1.92	0.0046
15	DSSN	0.22	1.86	0.0067
16	NPB	0.20	1.66	0.0216
17	LS	0.16	1.32	0.1380
18	NSTFN	0.16	1.29	0.1457

number of nodes per panicle (NNP), penultimate leaf width (PLL), lower glume length (SGL), number of nerves in the second glume (NNSG), distance between spikelets of the branches at the second node (DSSN), total number of primary branches per panicle (NPB), average length of the spikelets (LS) and average number of spikelets at the branches of the third and fourth panicle nodes (NSTFN). Eight traits were associated with floral morphology (FGW, LL, SGW, LAL, FGL, LW, SGL and NNSG), seven traits were directly associated with diaspore production, including panicle shape (NFS, NBFSN, NNP, DSSN, NPB, LS and NSTFN), and three variables were associated with vegetative, leaf and sheath characteristics (FLL, FSL and PLL).

Congruence between classifications from successive years

These same 18 traits were used to build a population pairs distance matrix for each year. The Z value

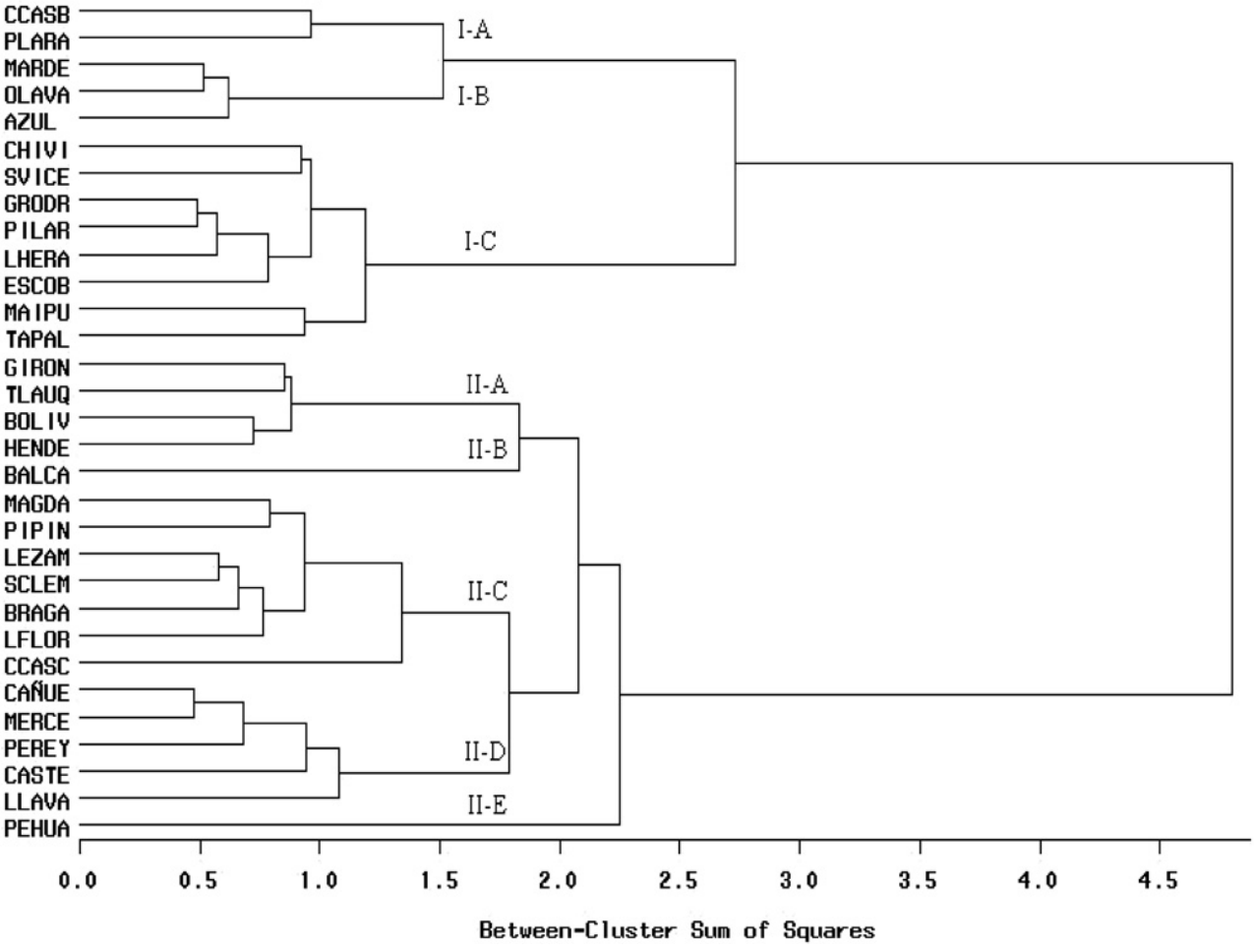

Fig. 1. Cluster analysis of a complete dataset based in 18 highly heritable traits of *Bromus caharticus*. Dendrogram built using Ward's minimum variance. Abbreviations as in Table 1.

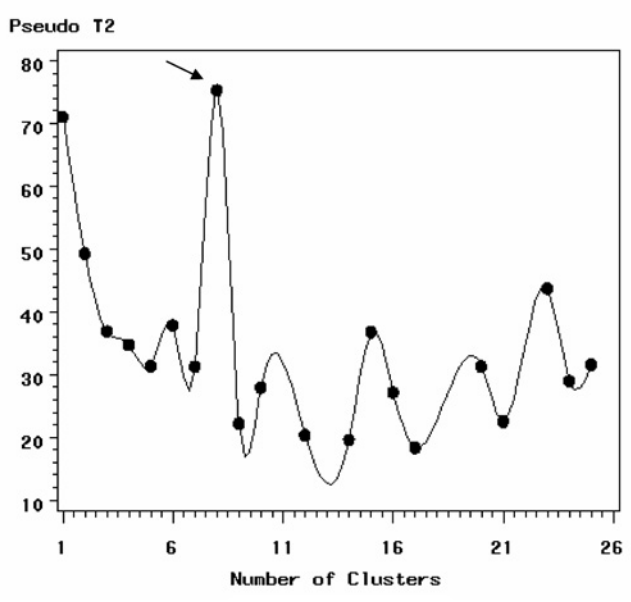

Fig. 2. Two dimensional plot of the pseudo t^2 statistic compared to the number of clusters obtained in the multivariate analysis of *Bromus catharticus*.

obtained for the comparison between the two year matrices was statistically non significant (*p*: 1), therefore, data from both experiments were pooled to obtain a unique pattern of classification.

Numerical taxonomy

The phenogram obtained from minimum variance clustering method showed the separation of the populations into eight groups distributed in two main clusters (Fig. 1). A local peak in the plot of pseudo t^2 statistic against the number of clusters confirmed that eight different clusters could explain the phenotypic variability (Fig. 2). Means, coefficients of variation (in percentage), and minimum and maximum values were calculated for each cluster. Statistical comparisons of means were carried out by one-way analysis of variance and a Tukey's test (Table 4). The eight discovered clusters were not significantly different for four reproductive (LAL, NNP, NPB and NSTFN) and for one vegetative (PLL) characters (Table 4).

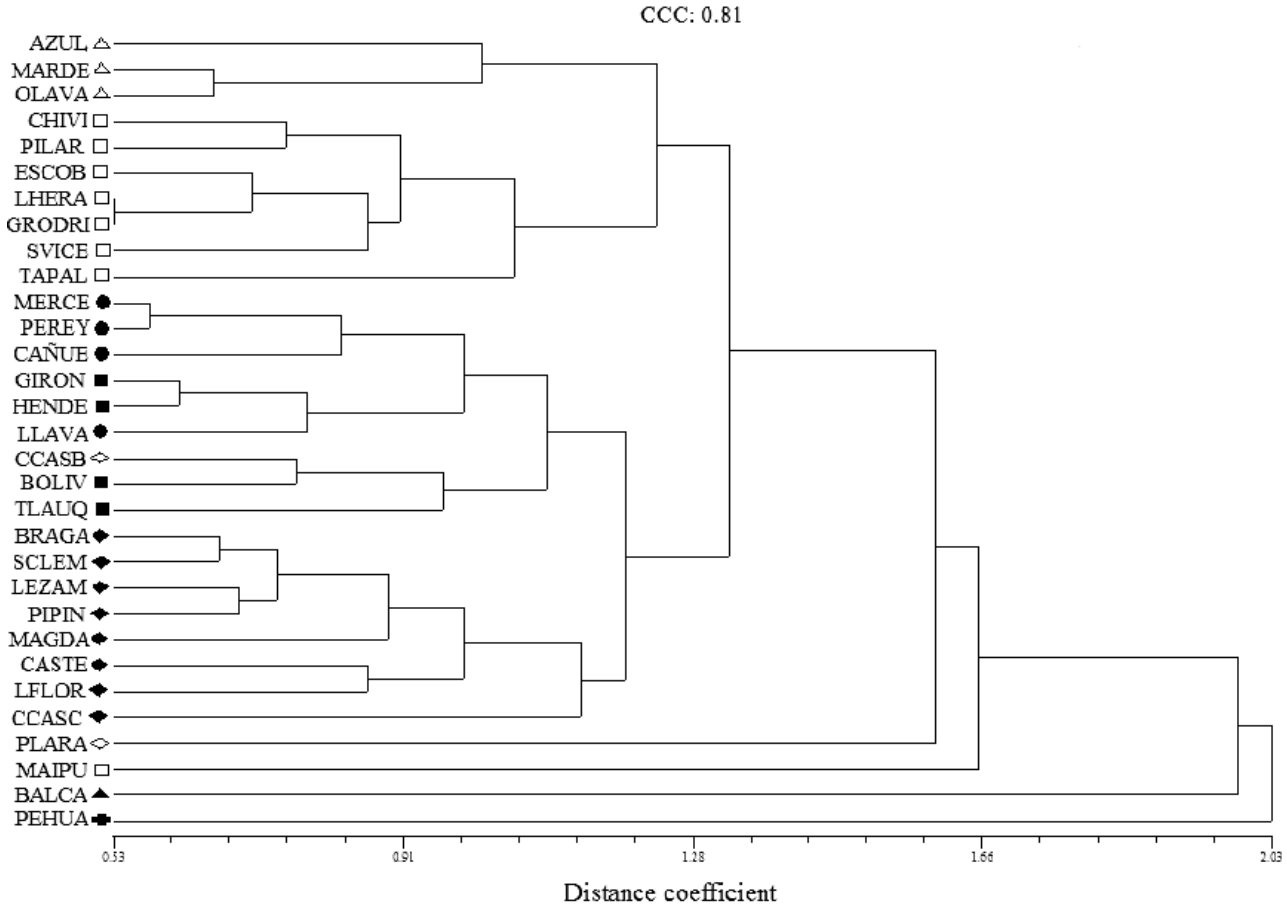

Fig. 3. Cluster analysis of a complete morphological dataset based in 18 highly heritable traits of *Bromus catharticus*. Dendrogram based on UPGMA clustering method. Population symbols represent the main group I (white) and II (black) and the eight clusters discovered by Minimum variance method. Cluster I-A (◇), Cluster I-B (△), Cluster I-C (□), Cluster II-A (■), Cluster II-B(△), Cluster II-C (◆), Cluster II-D (●), Cluster II (E) (+). Abbreviations as in Table 1.

Table 4. Means, coefficient of variation in percentage (CV %), minimum (MIN) and maximum (MAX) values per cluster for the selected traits of *Bromus catharticus*. F statistic test of the ANOVA (F) and *p* value. Clusters with the same letter are not significantly different at the 5 % level of Tukey's test. Significance levels: ns: *p>0.05*; *: *p<0.05*; **: *p<0.01*; Abbreviations as in Table 2.

CLUSTER	TRAITS	FGW (cm)	NFS	LL (cm)	SGW (cm)	FLL (cm)	LAL (cm)	NBFSN	FGL (cm)	FSL (cm)	LW (cm)	NNP	PLL (cm)	SGL (cm)	NNSG	DSSN (cm)	NPB	LS (cm)	NSTFN
I-A	MEAN	0.84 A	6.73 B	1.88 B	0.58Á	21.17 B	0.41 A	2.64 A B	1.25 A B	12.55 B	1.25 A	6.94 A	24.46 A	0.96 A B	6.27 A B	0.96 A B	18.15 A	3.10 A B	6.54 A
	CV%	5.76	9.52	5.37	5.71	15.73	42.92	20.52	4.96	7.58	8.28	20.66	16.86	16.93	7.39	16.93	23.68	5.64	37.84
	MIN	0.7.4	5.57	1.74	0.51	16.73	0.19	2.06	1.14	10.74	1.03	4.75	19.68	0.75	5.3	0.75	11.62	2.87	3
	MAX	0.91	7.64	2.09	0.64	28.01	0.73	3.85	1.39	14.34	1.41	9	33	1.27	7.1	1.27	25	3.36	9.79
I-B	MEAN	0.81 A B	7.08 A B	1.88B	0.59 A	20.64 B	0.48 A	2.57 A B	1.25 A B	12.9 B	1.20 A B	6.6A	24.33 A	0.99 A	6.71 A	0.95 A B	17.72 A	3.16 A B	6.12 A
	CV%	5.32	10.2	3.96	6.55	10.04	36.56	19.86	6.02	6.85	5.65	22.77	15.04	7.42	13.15	11.80	22.76	9.37	36.76
	MIN	0.71	6.15	1.76	0.53	16.38	0.22	1.9	1.12	11.27	1.07	4.67	18.43	0.89	5.4	0.80	11.54	2.63	2.64
	MAX	0.91	9.08	2.08	0.63	23.86	0.76	3.44	1.47	14.76	1.31	9.1	29.7	1.22	8.5	1.22	23	4.06	9.35
I-C	MEAN	0.82 A	6.82 B	1.96 A	0.58 A	22.28 A B	0.49 A	2.88 A	1.27 A	13.5 A B	120 A B	6.66 A	26.21 A	1.0 A	6.34 A B	0.99 A	18.8 A	3.19 A B	6.61 A
	CV%	4.81	8.41	4.22	6.34	12.41	38.84	23.98	4.26	5.6	5.54	21.26	17.14	5.46	10.37	12.64	23.04	6.17	33.9
	MIN	0.7	5.5	1.74	0.49	16.93	0.21	2	1.15	11.76	1.08	4.44	18.08	0.87	5.3	0.79	11.5	2.73	2.9
	MAX	0.91	8.82	2.18	0.67	29.99	0.88	4.5	1.38	15.22	1.36	8.8	36.84	1.14	7.9	1.42	26	3.81	10.44
II-A	MEAN	0.80 B C	6.58 B	1.86 B	0.57 A B	21.98 B	0.43 A	2.89 A	1.2 B	12.83 B	1.18 A B	6.65 A	25.78 A	0.93 B	6.26 A B	0.93 B	19.24 A	3.04 B	6.98 A
	CV%	5.8	10.06	5.7	7.2	12.16	46.58	23.71	6.78	5.93	5.89	20.33	15.38	8.49	9.09	14.32	23.52	6.73	33.99
	MIN	0.66	5.5	1.64	0.46	16.33	0.2	2	1.04	11.47	1.05	4.57	19.66	0.8	5.1	0.72	12.3	2.64	3.32
	MAX	0.87	8.58	2.07	0.65	27.59	0.76	4.18	1.38	14.62	1.35	8.7	34.34	1.14	7	1.25	29	3.62	10.3
II-B	MEAN	0.77 B C	6.46 B	1.95 A	0.53 B	21.96 B	0.51 A	3.28 A	1.19B	13.58 A B	1.17 A B	7.15 A	2S.54 A	0.91 B	5.69B	1.00 A	2139 A	3.08 A B	8.09 A
	SD	4.88	8.91	4.76	5.05	13 A1	30.25	21.92	3.94	4.7	7.19	20.7	16.02	5.86	6.12	12.82	20.51	6	28.48
	MIN	0.71	5.5	1.85	0.49	18.95	0.33	2.35	1.13	12.89	1.06	5.5	21.77	0.86	5.2	0.76	15.7	2.76	4.95
	MAX	0.81	7.16	2.14	0.58	27.38	0.7	4.17	1.26	14.73	1.3	9	34.41	1	6.2	1.12	27.92	3.39	10.71
II-C	MEAN	0.79 B C	7.24 A	1.88 B	0.56 B	21.16 B	0.44 A	2.46 B	1.21 B	13.21 B	1.19 A B	6.72 A	24.92 A	0.94 B	6.22 B	0.92 B	17.51 A	3.17 A B	6.46 A
	SD	4.94	9.31	4.58	6.44	10.17	36.11	17.22	5.88	7	6.39	21.39	15.11	6.76	9.44	10.30	19.19	7.16	33.91
	MIN	0.68	5.91	1.68	0.47	16.7	0.21	1.85	1.06	10.86	1.04	4.67	18.03	0.82	5.1	0.71	10.8	2.54	2.75
	MAX	0.86	8.92	2.04	0.64	28.76	0.77	3.35	1.36	15.04	1.33	9	35.63	1.07	7.6	1.15	23.8	3.72	10.6
II-D	MEAN	0.78 B C	6.96 B	1.92 A B	0.56B	21.84 B	0.52 A	2.71 AB	122B	13.19 B	1.16 B	6.52 A	25.88 A	0.95 B	635 A B	0.93 B	18.18 A	3.17 A B	6.75 A
	CV%	6.17	10.15	4.41	6.11	12.81	38.37	22.21	5.2	5.85	6.48	20.97	18.15	6.96	10.64	12.62	23.09	6.58	39.68
	MIN	0.68	5.11	1.74	0.48	16.29	0.23	2	1.08	11.3	1.04	4.56	17.66	0.82	5.22	0.76	10.78	2.76	2.33
	MAX	0.86	8.5	2.07	0.62	28.03	0.85	4	1.35	14.69	1.32	8.67	35.29	1.09	8.1	1.24	24.44	3.86	11.28
II-E	MEAN	0.76 C	6.95 A	1.88 B	0.55 B	25.2 A	0.51 A	2.32 B	1.18 B	14.22 A	1.20 A B	6.26 A	29.49 A	0.91 B	6.42 A B	0.89 B	17.54 A	3.29 A	7.47 A
	SD	8	10.19	4.66	8.48	18.99	41.66	10.26	3.74	6.72	7.49	15.95	20.87	5.44	15.8	9.88	14.52	8.99	36.94
	MIN	0.68	5.9	1.77	0.48	16.51	0.3	2.05	1.1	12.44	1.04	5.12	19.18	0.83	5.1	0.75	15.22	2.84	4.55
	MAX	0.83	8.32	2.01	0.63	31.12	0.84	2.69	1.23	15.36	1.28	7.44	37.05	0.98	7.7	1.01	21.38	3.83	11.69
F test		6.93	4.36	6.91	4.89	3.34	1.31	4.44	6.58	5.76	2.11	0.4	1.84	6.82	2.55	2.37	1.46	2.23	0.9
P value		**	**	**	**	**	ns	**	**	**	*	ns	ns	**	*	*	ns	*	ns

Characters FGW, SGW, FGL, SGL, LW, LL and DSSN showed significant differences between Cluster I and II ($p < 0.01$) (results not shown). The rest of significant variables allowed to discriminate between subgroups inside the two principal clusters (group I and group II) (Table 4).

UPGMA clustering method, and PCA ordination method were used to distinguish between data-dependent and method-dependent features of the results (following Dickinson & Phipps, 1985). The KMO test gave a value of 0.84, which indicated an adequate plant sampling further supporting the accuracy of the PCA analysis (Almeida Piñeiro de Carvalho & al., 2004). Populations scores were projected on the three principal components (Fig. 4) and factor loadings for these components were calculated (Table 5). The first three components were significant and their cumulative percentages accounted for 66.3% of the total variability. PCA allowed us to identify the most important traits (Table 5). Reproductive characters such as NSTFN, FGW, SGW and LW contributed most to the separation of accessions along PCA axis 1 (26.8 %), while characters LL, NBFSN, FGL and SGL contributed most to the separation along axis 2 (22.9 %). Moreover, LS and NNP were the most important attributes along the third component (16.5%). However, some of these main attributes selected by PCA showed non-significant differences between clusters when the Tukey test was used (e.g. NNP and NSTFN) (Table 4).

The Euclidean distance-based UPGMA dendrogram showed a good fit to the model (CCC = 0.81). Both methods, UPGMA and PCA (Figs. 3 and 4), produced similar results and differed slightly from the grouping structure obtained by minimum variance clustering method (Fig. 1). The groups I-B, I-C and II-C were recovered in all analyses. The group I-A from the dendrogram built using Ward's minimum variance (Fig. 1) was dismounted and its population CCACB and populations of the groups II-A and II-D were joined together in the dendrogram based on UPGMA (Fig. 3). This dendrogram clearly separated

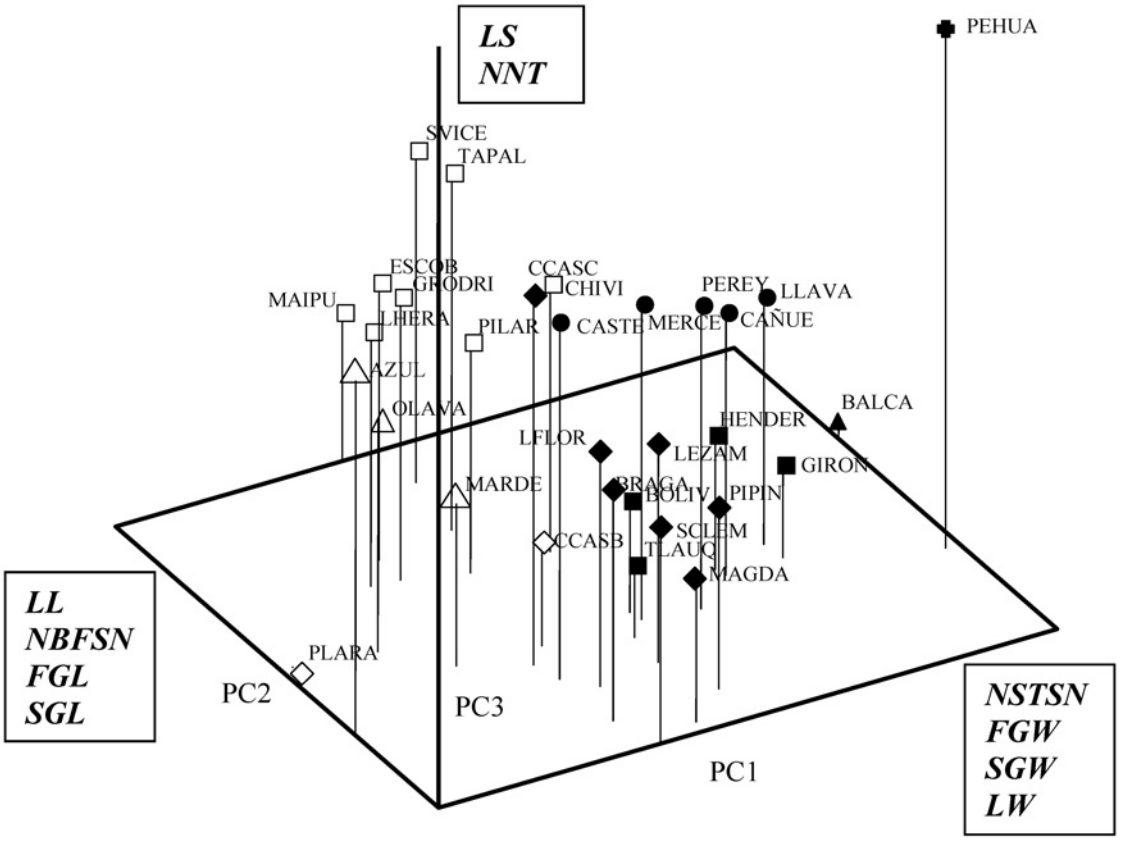

Fig. 4. Three-dimensional plot of the PCA performed with the complete morphological data set of *Bromus catharticus*. Variables with the highest loading for each component were inserted into squares. The three principal components of the correlation matrix accounted for 26.8%, 22.9% and 16.5% of the total variance, respectively. Population symbols represent the main group I (white) and II (black) and the eight clusters discovered by Minimum variance method. Cluster I-A (◇), Cluster I-B (△), Cluster I-C (□), Cluster II-A (■), Cluster II-B(▲), Cluster II-C (◆), Cluster II-D (●), Cluster II (E) (+). Abbreviations as in Table 1.

the populations PLARA, MAIPU, BALCA and PEHUA from the rest of the clusters.

Populations were separated into two main groups in the dendrogram based on Ward's minimum variance (Fig. 1). Group I includes populations with long and wide glumes and lemmas (Table 4) whereas Group II includes populations with short and narrow glumes and lemmas. The two main groups (I and II) also showed different panicle morphotypes: lax branching *versus* condensed branching (with the exception of BALCA, Group II-B, that also had lax branches). The majority of the populations in group I were collected from Ondulating Pampa and Interhill Pampa (Fig. 5). With the exception of group II-D (the northerly populations) and the population BALCA (group II-B), populations included in group II were associated with Sandy Pampas and Depressed Pampas. Sandy soils are common in the Sandy Pampa and in some Depressed Pampa areas near the drainage of the Río Salado or near the coast of the Río de la Plata (Tricart, 1973).

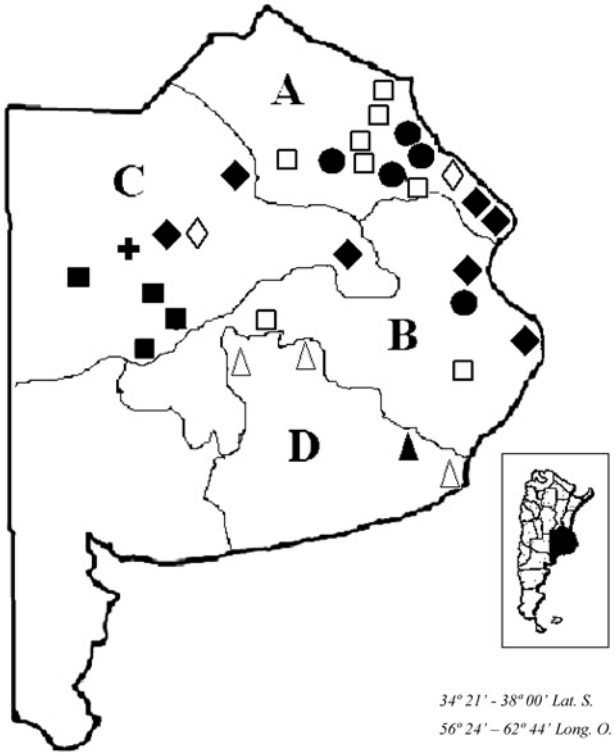

Fig. 5. Geographic location of the sampled populations of *Bromus catharticus*; the eight clusters of the dendrogram are indicated on the map. Symbols of the populations represent two main groups I (white) and II (black); and the eight clusters discovered by Minimum variance method. Cluster I-A (◇), Cluster I-B (△), Cluster I-C (□), Cluster II-A (■), Cluster II-B(▲), Cluster II-C (◆), Cluster II-D (●), Cluster II (E) (+). Abbreviations as in Table 1.

The geographic distribution of our sampled populations revealed that those populations sampled near one another tended to form alliances. For example, individuals from MARDE, OLAVA and AZUL populations (Cluster I-B) were all collected from the Interhill Pampas. Cluster I-C GRODRI, PILAR, LHERA, ESCOB, SVICE and CHIVI populations and cluster II-A populations were collected in the Ondulating Pampa and the Sandy Pampa, respectively (Fig. 5).

In the multivariate linear regression analysis both the F statistic and the R^2 indicated that the PC2 had a good fit to the lineal model. Climatic variables related to absolute maximum temperature (ºC) and relative humidity (%) showed a significant effect on the population projection scores on the PC2 (Table 6). The analysis also revealed that no environmental variables had a significant contribution to the model in the components 1 and 3.

Discussion

The aim of this research was to classify *Bromus catharticus* populations using highly heritable traits since they are under strong genetic control and might be less affected by environmental factors (Amurrio & al., 1995). The proved congruence between classifications obtained in two consecutive years for the two experimental environments confirmed that the use of highly heritable traits is apparently an efficient tool

Table 5. Contribution of the 18 selected morphological traits of *Bromus catharticus* to the main PCA components. Percentage of variability explained by the three principal components is indicated (PC1, first component; PC2, second component; PC3, third component). Abbreviations as in Table 2.

	PC1 (26.8 %)	**PC2** (22.9%)	**PC3** (16.5%)
FLL	0.60	0.33	0.44
FSL	0.34	0.33	0.61
PLL	0.63	0.30	0.43
NNP	– 0.03	0.11	– 0.74
NPB	0.48	0.63	– 0.46
NBFSN	0.26	0.77	– 0.36
NSTFN	0.84	0.25	– 0.28
NFS	– 0.37	– 0.52	0.47
LS	– 0.25	0.01	0.77
DSSN	– 0.15	0.61	– 0.07
FGL	– 0.52	0.71	0.17
SGL	– 0.62	0.68	0.28
NNSG	– 0.46	0.03	0.27
LL	0.01	0.78	0.32
FGW	– 0.7	0.47	– 0.26
SGW	– 0.68	0.36	0.02
LW	– 0.65	0.39	– 0.30
LAL	0.27	0.43	0.39

for removing non-genetic components when a replication trial (multi-locations or multi-years) is used.

Both univariate and multivariate methods allowed discovering the most useful attributes to discriminate groups. Several reproductive variables related to the floral structures (FGW, SGW, LW, FGL, SGL, NNSG, LS, LL) and panicle shape (NSTFN, NBFSN, DESN, NFS) and some morphological vegetative traits (FLL and FSL) were selected by ANOVA and PCA. This might indicate that some genes associated to fitness are possibly segregating as response to differential selection pressure. By contrast, only two vegetative traits associated to flag leaf were selected as classificatory variables. Aulicino & Arturi (2002) pointed out a narrow genetic variability for such attributes due to plastic responses. Since selection does not seem to affect plastic traits (Anderson, 1989), it is expected that such traits had not weight in systematic studies.

The ranges of variation of all quantitative morphological characters showed overlapping distributions among clusters, suggesting an outcrossing rate larger than that reported in the literature (García & Arturi, 1992). Previous systematic studies of the *B. catharticus* complex showed controversial results (Peterson & Planchuelo, 1998; Massa & al., 2004). These authors also found overlapping variation among infraspecific discontinuities (subspecies) based on only one quantitative trait, the length of lemmatal awn. However, infraespecific differentiation was easily recognized when DNA fragments and other qualitative attributes were used (Massa & al., 2001, Peterson & Planchuelo, 1998). Although the lemmatal awns showed non-significant differences between groups, the range of variation of this traits encountered in this study was similar to that described by Massa & al. (2004) in *B. catharticus* Vahl subsp *stamineus*. However, all the groups had minimum values < 3 mm long, coinciding with *B. catharticus* var *catharticus* Planchuelo & P.M. Peterson. It is possible that these subespecific discontinuities found by others authors are associated to large distances between collection sites. The application of a systematical sampling following an environmental gradient could be more efficient in discovering variation patterns.

Table 6. Multivariate lineal regression analysis calculated between environmental variables and the *Bromus catharticus* population's projections scores on the resulting PC axes (PCA1, PCA2 and PCA3). F statistic test. Significance levels: ns, not significant; *, $p < 0.05$; **, $p < 0.01$.

Environmental variables	PCA1	PCA2	PCA3
Model	0.33 ns	4.55*	1.08 ns
Average maximum temperature (ºC)	0.65 ns	0.15 ns	1.37 ns
Average minimum temperature (ºC)	0.19 ns	0.58 ns	0.00 ns
Absolute maximum temperature (ºC)	0.50 ns	5.80*	0.01 ns
Absolute minimum temperature (ºC)	0.00 ns	0.41 ns	0.05 ns
Average temperature (ºC)	0.31ns	0.52 ns	2.10 ns
Average precipitation (mm)	0.61 ns	1.59 ns	2.20 ns
Relative humidity (%)	0.02 ns	5.31 *	1.55 ns

In spite of the low levels of morphological variability observed, dendrograms and graphics confirmed phenotypic diversity. As suggested by Dickinson & Phipps (1985), the level at which such discontinuities should be recognized taxonomically is another matter. However, given that only highly heritable traits were used, we can confirm that populations grouped in the same cluster are more genetically similar to one another and that these groups reflect genetic divergence as portrayed by morphological characteristics.

Although the group structure depends on the sorting algorithm used, some groups were constant in all analyses (groups I-B, I-C and II-C) confirming their phenetic integrity. The populations PLARA, MAIPU, PEHUA and BALCA showed an outlier behaviour due to their extreme variation or to their shared traits with different groups. Since they were collected from marginal locations, they could represent samples of surrounding new groups.

Based on the above mentioned results, we propose a non-formal taxonomic treatment of the groups and recognize the discontinuities as morphotypes. We recognized the differentiation of 7 morphotypes based on those traits that were selected by PCA and ANOVA, simultaneously.

A key to aid in the identification of the morphotypes of *Bromus catharticus* Vahl is presented below.

Dichotomous key to the morphotypes

1. Upper glume > 8 cm wide, ⩾ 1.25 cm long; Lower glume > 5.8 cm wide, ⩾ 0.98 cm long 2
   Upper glume < 8 cm wide, < 1.25 cm long; Lower glume < 5.8 cm wide, < 0.98 cm long 4
2. Lemma < 1.90 cm long; flag leaf < 24.5 cm long; flag leaf sheath < 13 cm long 3
   Lemma > 1.95 cm long; flag leaf > 25.5 cm long; flag leaf sheath > 13.5 cm long ..... Morphotype A
3. Number of flower/ spikelets > 7 ..... Morphotype B
   Number of flower/ spikelets < 7 ..... Morphotype C
4. Distance between spikelets < 0.94 cm long (condensed branches); Number of primary branches at the first and second nodes < 2.8 5
   Distance between spikelets > 0.99 cm long (lax branches); Number of primary branches at the first and second nodes > 3 ..... Morphotype D
5. Number of flowers/ spikelets > 7 6
   Number of flowers/ spikelets < 7 ..... Morphotype E
6. Flag leaf > 25 cm long; Penultimate leaf > 29 cm long; flag leaf sheath > 14 cm ..... Morphotype F
   Flag leaf < 25 cm long; Penultimate leaf < 29 cm long; flag leaf sheath < 14 cm ..... Morphotype G

It was not possible to distinguish between MAIPU and the cluster I-C and between cluster II-A and II-D. To clarify this question, a correspondence between morphotypes and groups built with the minimum variance clustering method is cited as following: Morphotype A: MAIPU and the group I-C (CHIVI, PILAR, ESCOB, LHERA, GRODRI, SVICE, TAPAL). Morphotype B: group I-B (AZUL, MARDE, OLAVA). Morphotype C: PLARA. Morphotype D: BALCA (group II-B). Morphotype E: CCASB and the groups II-A (MERCE, PEREY, CAÑUE, LLAVA) and II-D (GIRON, HENDE, BOLIV, TLAUQ). Morphotype F: PEHUA (group II-E). Morphotype G: Group II-C (BRAGA, SCLEM, LEZAM, PIPIN, MAGDA, CASTE, LFLOR, CCASC).

Pattern of classification associated with the environment

We found differential panicle forms that could have an adaptive importance. The condensed branches would offer better protection for reproductive parts, especially in dry or saline habitats. Szpiniak & al. (1995) pointed out that drought and salinity might be important factors to select for shorter and narrower *B. catharticus* panicles.

The tendency for populations collected from nearby sites to group near to each other might be explained by the hypothesis of a common genetic origin or by a similar environmental response. In contrast of that, Morphotype G (cluster II-C) contains very geographically distant populations that are grouped together, possibly in response to the existence of interrupted areas or patches with a similar habitats, that are found in the depression of the Río Salado drainage (Sandy and Depressed Pampas) [Fig. 5]. In contrast populations CCASC and CCASB, sampled from nearby one another, were placed in different clusters by the third component. When using only the first and second components these same populations formed a single cluster (Fig. 3). This indicates that some variables such as spikelet length (LS) and number of nodes per panicle (NNP) could be selected by micro rather than macro environmental conditions.

Our collecting locations cover a gradient of humidity and temperature that diminishes from the Northeast to the Southwest of Buenos Aires province. We suggest that lemma and glume length, and the number of branches, which are the most important variables in the second component, might have a role in the adaptation of populations to this gradient. The fact that temperature and humidity were significantly correlated with the scores of the populations on PC2 (Table 6) seems to support this idea. Our conclusion was further confirmed since the northernmost population (morphotype A) presented the largest lemmas and glumes and numerous branches (Table 4). Coincidently, Smith (1981) and Sales (1994) associated the variation in lemmas, glumes and fruit size with floatability and water availability.

When a characteristic is genetically fixed and is correlated with ecological and physical factors of the habitat it is defined as an ecotype (Bradshaw, 1965). The morphotypes that are associated with environmental factors could be recognized as *B. catharticus* ecotypes. They seemed to be determined by microenvironment factors such as the landscape, which is mainly defined by topography and soil geomorphology, and some climatic factors such as the temperature and humidity. These results suggest that new germplasm would be found if we extend the present sampling to include all the geographic, climatic, and ecological variation found across the native distribution area of *B. catharticus*.

In conclusion, a patchy variation model seems to best explain the distribution of populations as measured by these 18 morphological heritable traits. Our data supports the classification or grouping of morphologically and genetically similar populations collected at neighboring areas with similar environmental conditions. As a consequence, the pattern of classification reflects the geographical origin, although there is still some noise with this model.

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