Genetic diversity among varieties of the native forage grass *Trichloris crinita* based on AFLP markers, morphological characters, and quantitative agronomic traits

Pablo F. Cavagnaro, Juan B. Cavagnaro, José L. Lemes, Ricardo W. Masuelli, and Carlos B. Passera

Abstract: We assessed the genetic diversity in *Trichloris crinita* (Poaceae) varieties from South America, using AFLPs, morphological characters, and quantitative agronomic traits. Owing to the importance of this species for range grazing, we first characterized the varieties based on forage productivity. Biomass production varied 9 fold among the materials evaluated. Analysis of AFLP fingerprints allowed the discrimination of all varieties with a few selected primer combinations. Pair-wise genetic similarities, using marker data, ranged from 0.31 to 0.92 (Jaccard coefficients). Marker-based unweighted pair group method with arithmetic averaging (UPGMA) cluster analysis did not show geographical clustering, but rather grouped the varieties according to their biomass production. We identified 18 markers associated with biomass production, of which 8 showed complete correlation (r = 1.00) with this trait. These DNA markers can be used to assist selection for high forage productivity in *T. crinita*. Cluster analysis using morphological and quantitative characters revealed 4 distinct groups of varieties, clearly separated according to their biomass yield. The variables foliage height and basal diameter were strongly correlated with biomass production and these phenotypic markers can be used to select productive plants. The relations among the varieties based on AFLP data were significantly correlated with those based on agronomic and morphological characters, suggesting that the 2 systems give similar estimates of genetic relations among the varieties.

Key words: AFLP fingerprinting, genetic diversity, *Trichloris crinita*, morphology, biomass production.

Résumé : Les auteurs ont mesuré la diversité génétique au sein de variétés du *Trichloris crinita* (hordées) d'Amérique du Sud à l'aide de marqueurs AFLP et de caractères morphologiques ou agronomiques. En raison de l'importance de cette espèce au sein des prairies fourragères, les variétés ont d'abord été caractérisées sur la base de leur productivité en fourrage. La production de biomasse variait par un facteur 9 au sein du matériel évalué. Une analyse des empreintes AFLP a permis de distinguer toutes les variétés à l'aide de quelques paires d'amorces choisies. Les similarités génétiques calculées sur la base des marqueurs variaient entre 0,31 et 0,92 (coefficient de Jaccard). Une analyse de groupement UPGMA, fondée sur les marqueurs, n'a pas montré de groupement sur des bases géographiques, mais plutôt en fonction de leur production de biomasse. Les auteurs ont identifié 18 marqueurs associés à la production de biomasse dont huit montraient une corrélation parfaite (r = 1,00) avec ce caractère. Ces marqueurs moléculaires pourront servir en sélection assistée pour une productivité fourragère élevée chez le *T. crinita*. Une analyse de groupement sur la base des données morphologiques et agronomiques a révélé quatre groupes distincts de variétés, clairement séparés en fonction de leur rendement en biomasse. La hauteur du feuillage et le diamètre basal sont deux variables qui étaient fortement corrélées avec la production de biomasse et ces marqueurs phénotypiques peuvent servir à la sélection de plantes productives. Les relations entre les variétés sur la base des données AFLP étaient significativement corrélées avec celles fondées sur les données agronomiques et morphologiques. Ceci suggère que les deux systèmes fournissent des estimés semblables des relations génétiques entre variétés.

Mots clés : empreintes AFLP, diversité génétique, *Trichloris crinita*, morphologie, production de biomasse.

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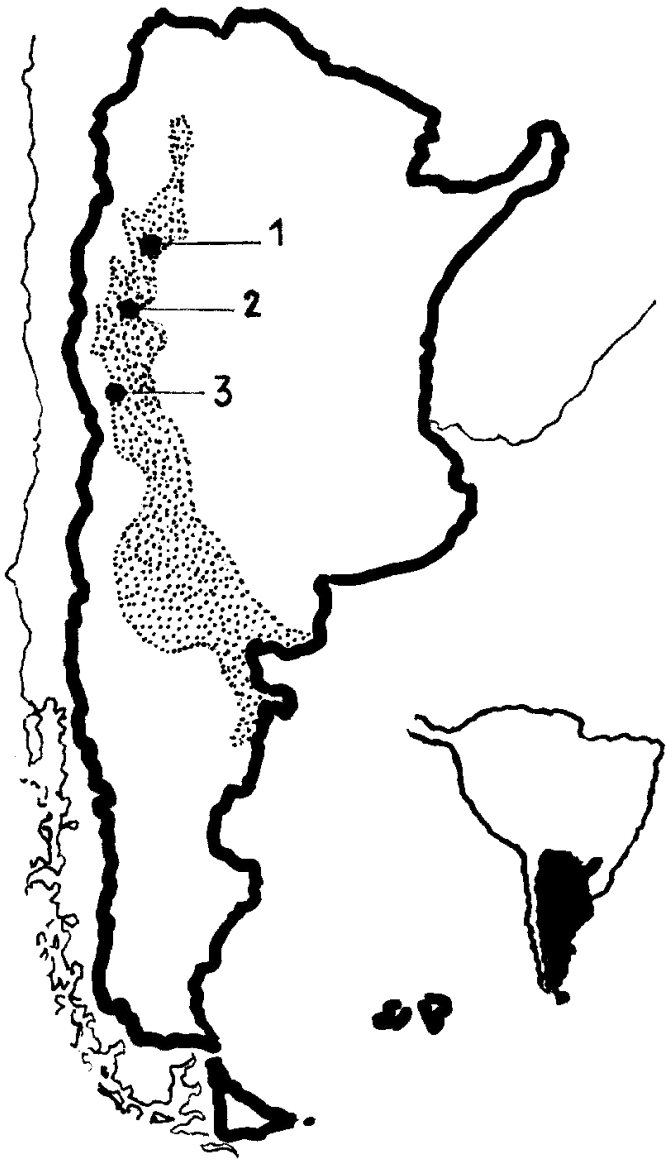
Introduction

Trichloris (Poaceae, Chloridoideae) is a native American genus that grows in arid and semiarid conditions in 2 disjunctive areas of the American continent: the south of North America, including north of Mexico and south of the United States, and the central and southern regions of South America, including Bolivia, Argentina, Chile, Uruguay, and Paraguay (Nicora and Rúgolo de Agrasar 1987). The only 2 species described, *T. crinita* and *T. pluriflora*, can be distinguished because *T. crinita* has lemmas with 3 awns of the same length; in *T. pluriflora*, the central awn is twice as long as the lateral awns (Cabrera 1970). Karyologically, both species have the same basic chromosome number ($n = x = 10$), but differ in ploydy levels, *T. crinita* being a tetraploid ($2n = 4x = 40$) and *T. pluriflora* a hexaploid ($2n = 6x = 60$) (Fedorov 1969). Polyploidy is highly common in the Chloridoideae, with more than 90% of the species being polyploids (Roodt and Spies 2003). Although the mode of reproduction of *T. crinita* has not been fully demonstrated, circumstantial evidence indicates that this species is either apomitic or highly autogamous. The absence of phenotypic segregation in 23 varieties of *T. crinita*, during several generations under open pollination condition (Greco and Cavagnaro 2002) and the lack of AFLP polymorphisms observed among plants of the same varieties (P.F. Cavagnaro, unpublished data), indicate that these materials do not intercross. The Chloridoideae subfamily comprises an unusually large number of apomictic taxa including several species of the genus *Chloris* (Brown and Emery 1958), a close relative of *Trichloris* (Anderson 1974).

Trichloris spp. are important as both forage grasses and in revegetation programs (USDA–ARS, Germplasm Resources Information Network, http://www.ars-grin.gov). Range grazing is one of the few economical activities in arid and semi-arid regions and native grasses constitute the main forage resources in these ecosystems. *Trichloris crinita* (Lag.) Parodi, a C4 species, is one of the most important perennial native grasses in the west arid region of Argentina known as Monte (Cavagnaro 1988), mainly owing to its forage quality, palatability, and wide area of distribution (Waistein and González 1969; Roig 1971). Under natural conditions it behaves as a typical aestival species growing whenever soil water is available and the temperature is above 10 °C (Seligman et al. 1992). *Trichloris crinita* is particularly aggressive in clay soils, where it prevails over other grass species (Roig 1971). An experience in Monte showed that *T. crinita* is suitable for rangeland seeding. Passera et al. (1992) found that after shrub removal and sowing of a mixture of seeds of 7 native grass species, *T. crinita* and *Pappophorum caespitosum* behaved as "pioneer species" (species capable of quick establishment) covering the space from which shrubs were removed, and significantly increased the overall forage coverage and livestock grazing capacity. Under simulated intensive and frequent grazing, *T. crinita* was less affected than *P. caespitosum* (Cavagnaro and Dalmaso 1983).

The phytogeographical province of Monte is a north–south extended area located along the eastern base of the Andes mountains in Argentina (Fig. 1). It is a shrubland dominated by species of the genus *Larrea* interspersed with grasses and other herbaceous species (Cabrera 1976), as well as a few tree species, mostly of the genus *Prosopis.* A comprehensive description of the flora of this warm desert has been compiled by Orians and Solbrig (1977).

Fig. 1. The phytogeographical province of Monte (dotted area). The 3 experimental sites Catamarca (1), La Rioja (2), and Mendoza (3) are indicated.



Previous studies on *T. crinita* have mostly focused on ecophysiological aspects and its nutritional value as a forage species. However, very little is known regarding its intraspecific variability, since most works evaluated only a few ecotypes or varieties. Characterizing the genetic variability within *T. crinita* would improve its use both as a forage crop and for re-vegetation purposes.

A germplasm collection of *T. crinita* was created by the Instituto Argentino de Investigaciones de Zonas Aridas (IADIZA), Mendoza, Argentina. Plants included in the collection were selected on the basis of their different morphologies, thus they represent a broad and diverse sampling of the phenotypic variation present in Monte. The main

purpose for creating this collection was to characterize the materials based on their performances in agronomic traits of interest (e.g., biomass yield) and their morphology.

Morphological data are fast and easy to score, but they are environmentally influenced and the number of distinctive characters is rather limited. Quantitative agronomic traits, such us biomass production, can also serve to distinguish varieties. DNA markers are useful complements of morphological data and quantitative agronomic traits. Amplified fragment length polymorphism (AFLP) (Vos et al. 1995) has been successfully used for assessing genetic diversity and relatedness in a number of grasses (Puecher et al. 2001; Renganayaki et al. 2001; Wu et al. 2004).

Hereby, we report on the assessment of genetic diversity of *Trichloris crinita* varieties using AFLPs, morphological characters, and quantitative agronomic traits. The specific objectives were to estimate the genetic variability available within a collection of *T. crinita* from Monte region, generate molecular fingerprints that can be used as complements to morphological and agronomic characters, characterize *T. crinita* varieties on the basis of their biomass production across different environments, and identify markers associated with high and low biomass production.

It is hoped that the results from this study will be useful in the selection of *T. crinita* varieties for rangeland seeding strategies and for breeding purposes, as well as in the management of germplasms collections.

Materials and methods

Collection and evaluation of the *T. crinita* core germplasm

Plants with different phenotypes were collected from 48 natural *Trichloris crinita* populations dispersed throughout 350 000 km^2 of the Monte region (Fig. 1) and the seeds from each population were combined and introduced for maintenance at the IADIZA Germplasm Bank of native grasses, Mendoza, Argentina. Seeds from each original population were planted in the experimental field of IADIZA under a randomized block design, consisting of 4 blocks (each experimental unit being ~ 30 plants), and the plants were evaluated on the basis of morphological characters. In this first screening, 9 polymorphic morphological traits were considered. Seeds from 23 individual plants exhibiting unique phenotypes were collected and these single-plant seeds were sown the following year and the derived plants evaluated with the same criteria. At the end of the growing season, seeds were collected from each morphotype and planted the following year for a second cycle of morphological evaluations. This process was repeated each year for a period of 4 years.

Morphological and quantitative agronomic traits

Seeds from 20 of the 23 *T. crinita* morphotypes, evaluated at IADIZA as previously described, were obtained from the IADIZA germplasm collection. Individual seeds were sown in 250 cm^3 pots with sterile soil and plants were grown under greenhouse conditions until they had 5–6 leaves. At this stage, they were transplanted to experimental fields at 3 sites in the Monte ecosystem, where we evaluated their performance in quantitative traits of agronomic interest each year during a 3 year period. Ten plants of each variety were randomly distributed in each experimental field at a spacing of 0.80 m × 0.80 m. Plants were irrigated every 2 days during the 3 weeks after their transplantation; after this period, their water availability depended only on the natural rainfall at each site. A borderline of plants was used on the perimeter of the trial for isolation purposes. Details of the sites were as follows: (*i*) Catamarca, 28° 27′ S, 65° 39′ W, 519 m altitude; (*ii*) La Rioja, 30° 21′ S, 66° 18′ W, 452 m altitude; and (*iii*) Mendoza, 32° 53′ S, 68° 51′W, 827 m altitude. Mean annual temperature and total annual rainfall varied among the tree sites as follows: Catamarca, 20.5 °C, 458 mm; La Rioja, 19.8 °C, 460 mm; and Mendoza, 15.9 °C, 270 mm. At the end of each growing season, the following variables were measured in individual plants: biomass production, number of culms, foliage and culm height, basal diameter, and leaf blade length and width. For evaluation of biomass production, the aerial part of each plant was cut 12 cm above ground level according to Cavagnaro and Dalmasso (1983) and oven-dried at 65 °C until a constant mass was reached. Values were expressed as dry matter (measured in grams) per plant (DM/plant).

The data were analyzed by ANOVA procedure using the software STATGRAPHICS Plus v. 4.0 for Windows. The ANOVA model was a factorial with 3 factors (variety, site, and year) with interactions and a completely randomized design in the field. Means were compared by least-significant difference (LSD) test ($p \leq 0.05$). For biomass production the values of DM/plant were transformed to Log_{10} DM/plant before performing ANOVA analysis to fulfill the ANOVA assumptions.

DNA extraction and AFLP analysis

Seeds from 20 selected *T. crinita* varieties, evaluated for biomass production and morphological characters, were obtained from the germplasm bank at IADIZA. Seeds from a variety of *Chloris virgatum* were obtained from the same source. All plants were grown in a greenhouse for 5–6 weeks, and then equal amounts of fresh leaf tissue from 6–8 plants from each variety were harvested and bulked for DNA extraction. Total genomic DNA was isolated as described by Murray and Thompson (1980). The concentration and quality of the DNA extracts were estimated by measuring the 260 and 280 UV absorbance in a Pharmacia Gene Quant spectrophotometer (Pharmacia, Biotech, Columbus, OH) and the integrity was checked by agarose gel electrophoresis (Maniatis et al. 1982). Only samples with 260:280 ratios of 1.8–1.9 and no degradation were used for AFLP analysis.

AFLP reactions were carried out following the instructions supplied with the GIBCO-BRL Life Technologies AFLP™ kit (Invitrogen, Carlsbad, Calif.). For selective amplifications either an *Eco*RI or an *Mse*I selective primer was end-labeled with ^{33}P. The following touchdown PCR program was used in all selective amplifications: 2 min at 94 °C; 13 touchdown cycles at 94 °C for 30 s, 65 °C for 30 s (decreasing the temperature by 0.7 °C/cycle), and 72 °C for 60 s; and 25 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. PCR products were resolved on 6% *w*/*v* denaturing polyacrylamide gel electrophoresis in 1× Tris–borate–EDTA (TBE) buffer. Gels were dried and exposed to X-ray film overnight. A total of 24 selective primer pairs were screened and the generated amplicons were scored.

To search for AFLP markers associated with biomass production we considered a high and a low biomass class and selected markers that were present in the high biomass class and absent in the low biomass class. Two lower limits for the high biomass class were considered: 110 g DM/plant (including varieties 11, 16, 6, 19, 20, 10, and 3) and, following a more strict criterion, 150 g DM/plant (including only the 3 most productive varieties, 11, 16, and 6. The low biomass class included varieties with fewer than 75 g DM/plant.

Data analysis

For each AFLP primer pair combination, monomorphic and polymorphic bands were visually scored across 20 *T. crinita* and 1 *Chloris virgatum* varieties. The latter was included as a putative outgroup. Only clearly readable bands were included in the binary data matrix (i.e., 1 denoted presence of band, 0 denoted absence) and pair-wise similarities were calculated among the varieties according to the Jaccard coefficient (Sneath and Sokal 1973), using the NTSYSpc software, v. 2.02g (Rohlf 1998). The resulting similarity matrix was used to create a dendrogram by the unweighted pair group method with arithmetic average (UPGMA) procedure (Sokal and Michener 1958).

A cluster analysis was done to depict relations among 20 *T. crinita* varieties based on their morphological and quantitative data (Table 1). To minimize the scale effects before Euclidian distances were calculated, morphological and quantitative agronomic traits were standardized by subtracting the mean value from the observed value and dividing by the standard deviation. Euclidian distances among the varieties were calculated using NTSYSpc software and a dendrogram was obtained by the UPGMA method.

Cophenetic values were calculated from both AFLP- and phenotype-based dendrograms and compared with their respective similarity matrices to evaluate whether the data in the similarity matrices are well represented by the phenograms.

The correspondence between the cophenetic matrix of Euclidian distances and the cophenetic matrix of AFLP-based similarities was tested with the Mantel *Z* statistic (Mantel 1967) using the software NTSYSpc v. 2.02g.

Results

Evaluation of the *T. crinita* core germplasm collection

Morphological analysis of the progenies from 23 originally tested morphotypes showed no phenotypic segregation among plants within each family; all the individuals showed identical phenotype of the plant from which they were derived. This process of evaluation was repeated over 4 generations (4 years) and the descendant plants always showed the phenotype of the original mother plant. Based on these observations, we inferred that seed production in *T. crinita* may be the result of apomixis and not amphimixis. Another possibility is that this species could be highly autogamous.

Biomass production

ANOVA analysis for biomass production showed significant variation ($p \leq 0.001$) for all single factors (variety, site, and year). Significant variety × site interaction was found ($p \leq 0.001$). Figure 2 shows biomass yield of *T. crinita* varieties in the 3 study sites. Mean values in Catamarca were 4 times higher than in La Rioja (Fig. 2). Considering their overall performance over 3 sites and 3 years, the varieties mean biomass production varied almost 9 fold (Table 1). Varieties 14 and 11 had the lowest and highest value for this attribute with mean values of 22 and 189 g DM/plant, respectively (Table 1). Variety × year interaction was not significant ($p = 0.784$). Based on their biomass production, the varieties were classified into 3 categories: low (<75 g DM/plant), medium (between 75 and 110 g DM/plant), and high (>110 g DM/plant) production.

Marker analysis and fingerprinting

Twenty-four AFLP selective primers generated amplicons from 30 to 600 bp and most of the polymorphisms were observed in band sizes of 100 to 400 bp (Fig. 3). On average, each primer combination generated 77 (±24) fragments, of which 39 (±17) were polymorphic (Table 2). However, extensive variation in the number of polymorphic bands generated was found among different primer-pairs, ranging from 7 to 87 (Table 2). A total of 2014 clear unambiguous bands were scored across 20 *T. crinita* and 1 *Chloris* variety. The *T. crinita* subset accounted for 1855 fragments. Of these, 930 (50.1%) were polymorphic on at least 2 varieties (Table 2). Considering the *Trichloris* plus outgroup (*Chloris*) varieties, 1243 polymorphic bands were generated, representing 61.7% of the fragments.

All *T. crinita* varieties and the outgroup *Chloris virgatum* were genotyped by AFLP markers. Figure 3 shows profiles with primers eAAG–mCCA, where 17 selected markers allowed discrimination of 12 (60%) varieties. In this case, varieties 3, 17, 18, 20, 8, 16, 4, and 9 could not be resolved.

Markers associated with biomass production

Among the 930 polymorphic fragments scored, we identified 18 markers for which presence of a band was significantly associated ($p \leq 0.05$) with high biomass production (Table 3). Considering a biomass of 110 g DM/plant as the lower limit for the high biomass class, 4 markers were completely associated ($r = 1.00$) with biomass production. Four other markers showed a correlation of 0.87, whereas 1 marker was associated at 0.75 (Table 3).

Considering a biomass of 150 g DM/plant as the cutoff for high productivity, we identified 4 markers showing complete correlation ($r = 1.00$) with this trait. One AFLP was present in all the productive varieties and absent in the low-yield varieties, but was also present in one of the intermediate varieties ($r = 0.87$). Four other markers were correlated with biomass production at 0.76.

AFLP-based genetic diversity and relatedness

Excluding the outgroup *Chloris virgatum*, pair-wise genetic similarities among the varieties ranged from 0.31 (for varieties 4 and 8 and varieties 8 and 9) to 0.92 (between 4 and 9) and only 32% of the comparisons shared more than 65% of the AFLP markers (data not shown). The average similarity between *Trichloris* varieties and the outgroup *Chloris virgatum* was 0.37 and varied from 0.32 to 0.51 (for varieties 4 and 19, respectively).

A phenogram constructed on the basis of 930 AFLP data depicts relations among the varieties in Fig. 4. Comparison of cophenetic values, calculated from Jaccard's similarity

Table 1. Morphological and quantitative agronomic characters of 20 *T. crinita* accessions.

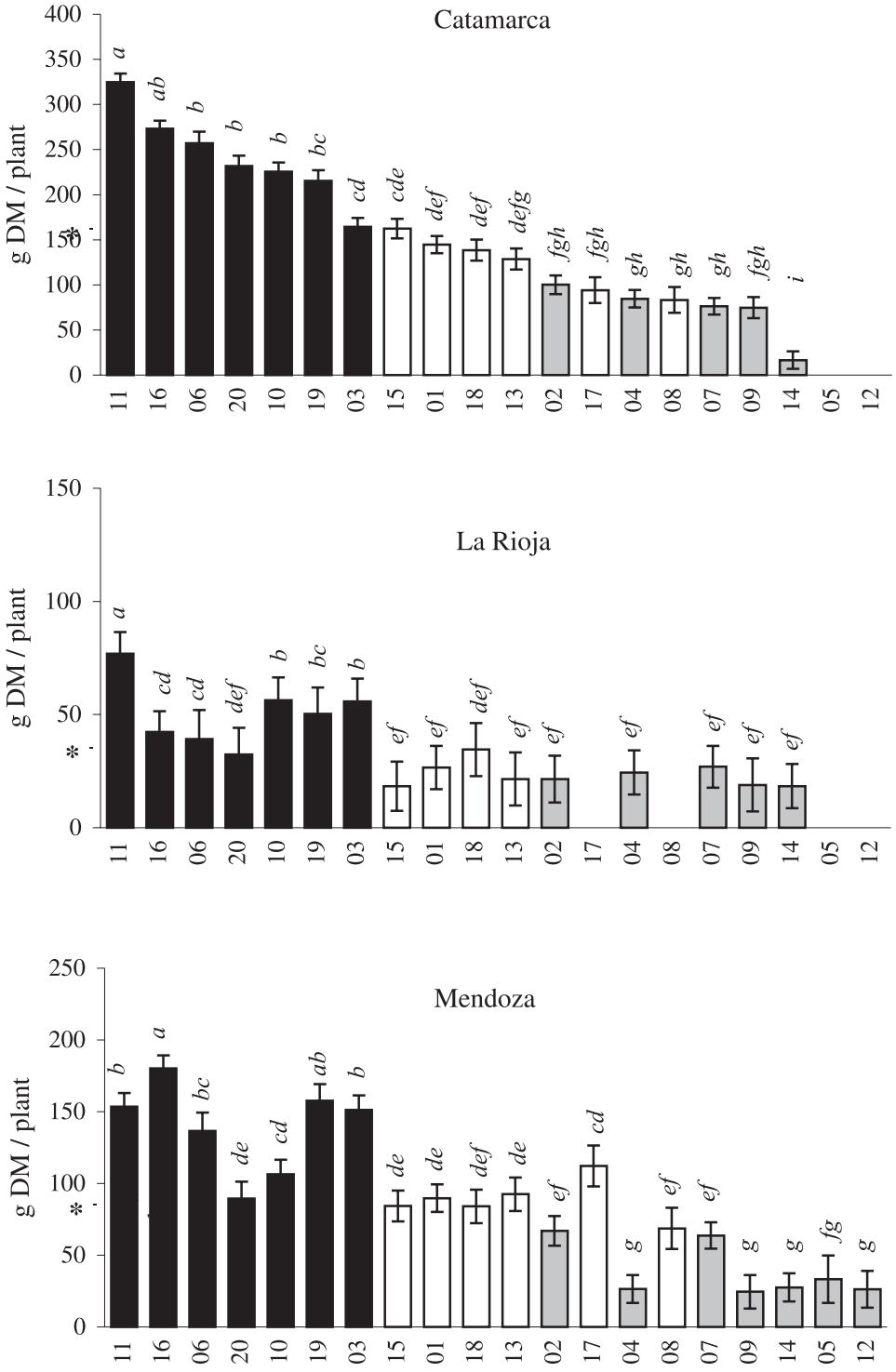
Acc. No.	Dry matter (g/plant)	Culm height (cm)	Foliage height (cm)	Basal diameter (cm)	Culm no.	TLB[a]	TLS[a]	TELS[b]	Color of spike	Color of inter-node	Color of node	Color of ligule	Aerial tillers[b]	Growth habit	Blade width (mm)	Blade length (mm)
1	88.1	78.1	16.4	12.4	52.4	–	+	–	R	R	R	R	0	SD	**5**	101
2	61.2	71.8	17.8	13.5	40.8	–	–	–	R	G	R	R	0	D	3	*75*
3	118.5	85.6	21.1	14.0	52.7	–	–	+	R	G	R	R	0	D	3.5	86
4	48.0	71.6	13.2	11.7	34.0	–	–	–	G	G	G	G	–	D	2.5	85
5	33.3**	53.4**	10.2**	*9.1***	*13.9***	+++	+++	+	G	G	R	R	0	SU	4.5	120
6	161.5	**95.1**	25.8	14.6	53.4	–	+++	+	R	G	R	R	0	SU	**5**	165
7	53.5	75.4	18.6	12.1	33.9	+	+	+	R	G	G	R	0	SU	5	175
8	74.9*	81.4*	18.6*	12.6*	25.2*	+	+	+	G	G	R	R	0	D	**5**	140
9	41.5	74.4	14.7	12.6	34.3	–	–	–	G	G	R	R	–	D	*2*	83
10	123.7	95.1	23.2	13.3	65.0	–	–	+	G	G	R	R	0	SD	**5**	144
11	189.5	91.8	**28.6**	16.1	71.2	–	+	+	R	G	R	R	0	SU	**5**	103
12	62.8**	*52.4***	*9.3***	9.2**	29.5**	+	++++	+	G	G	G	R	–	SU	3	93
13	88.2	85.0	21.9	11.4	65.3	–	–	+	R	R	R	R	0	U	4	95
14	*22.3*	63.6	12.4	10.3	46.1	–	+	–	G	G	G	G	–	SD	2.5	80
15	106.7	92.2	19.3	13.0	38.2	–	–	+	G	G	R	R	0	SU	4	110
16	169.3	84.3	27.4	14.5	53.7	–	–	+	G	G	R	R	0	SD	4	110
17	103.2*	91.5*	22.5*	13.2*	52.3*	–	–	+	G	G	R	R	0	SD	4.5	168
18	88.5	85.3	22.4	13.4	39.2	–	–	+	G	G	R	R	0	SU	4	140
19	142.1	82.2	27.1	14.9	64.6	–	–	+	G	G	R	R	0	SU	5	127
20	134.7	87.1	22.0	14.5	59.5	+	–	n.a.	G	G	G	n.a.	n.a.	SU	4	n.a.

Note: TLB, trichomes on the leaf blade; TLS, trichomes on the leaf sheath; TELS, trichomes on the edge of the leaf sheath; SD, semi-decumbent; SU, semi-upright; U, upright; D, decumbent; R, red; G, green; n.a., not available. Bold and italic numbers indicate the upper and lower extremes, respectively, of each quantitative character. Exceptions are indicated for means calculated from 3 years and 2 locations (*) and from 3 years and 1 location (**). Data from the variables dry matter, culm height, foliage height, basal diameter, and culm number are mean values from 3 years and 3 locations.

[a]Dashes (–) in this column indicate absence; single plus sign (+) indicates very scarce; ++, scarce; +++, medium density; ++++, highly dense.

[b]Dashes (–) in this column indicate absence; single plus sign (+) indicates presence.

Fig. 2. Biomass production of 20 *T. crinita* accessions evaluated in 3 locations (Catamarca, La Rioja, and Mendoza). Values are means of 3 y data (± standard error). Black, white, and grey bars represent accessions with high (> 110 g DM/plant), medium (between 75 and 110 g DM/plant), and low (< 75 g DM/plant) biomass yield, respectively, considering their overall performance in the 3 locations (Table 2). Bars with the same letters are not significantly different at $p \leq 0.05$, LSD test. Asterisk indicates mean value for the location.



matrix demonstrated a correlation of 0.94, indicating that the dendrogram was a very good representation of the data in the similarity matrix (Rohlf 1998).

By clustering the varieties with more than 65% similarity, 7 groups were revealed. Three major clusters designated I, II, and VII grouped 80% of the varieties. Groups III, IV, V, and VI were composed of only 1 variety each, being more distantly related (< 65% similarity) from each other and from the rest of the clusters. *Chloris virgatum* was clearly separated from the *Trichloris* group and formed the bottom branch of the tree.

Considering the 3 major groups, it is apparent that varieties

Fig. 3. Profiles of AFLP fingerprints (by duplicates) for 20 *T. crinita* (1–20) and 1 *Chloris virgatum* (21) accessions using the selective primer combination eAAG–mCCA. Fragment size is indicated on the left. Arrows indicate markers that allowed discrimination of 12 (60%) of the accessions. White arrows indicate cluster-specific markers. Cluster numbers are indicated on the left with bold (showing presence of a band) and regular (showing absence of a band) roman numerals.

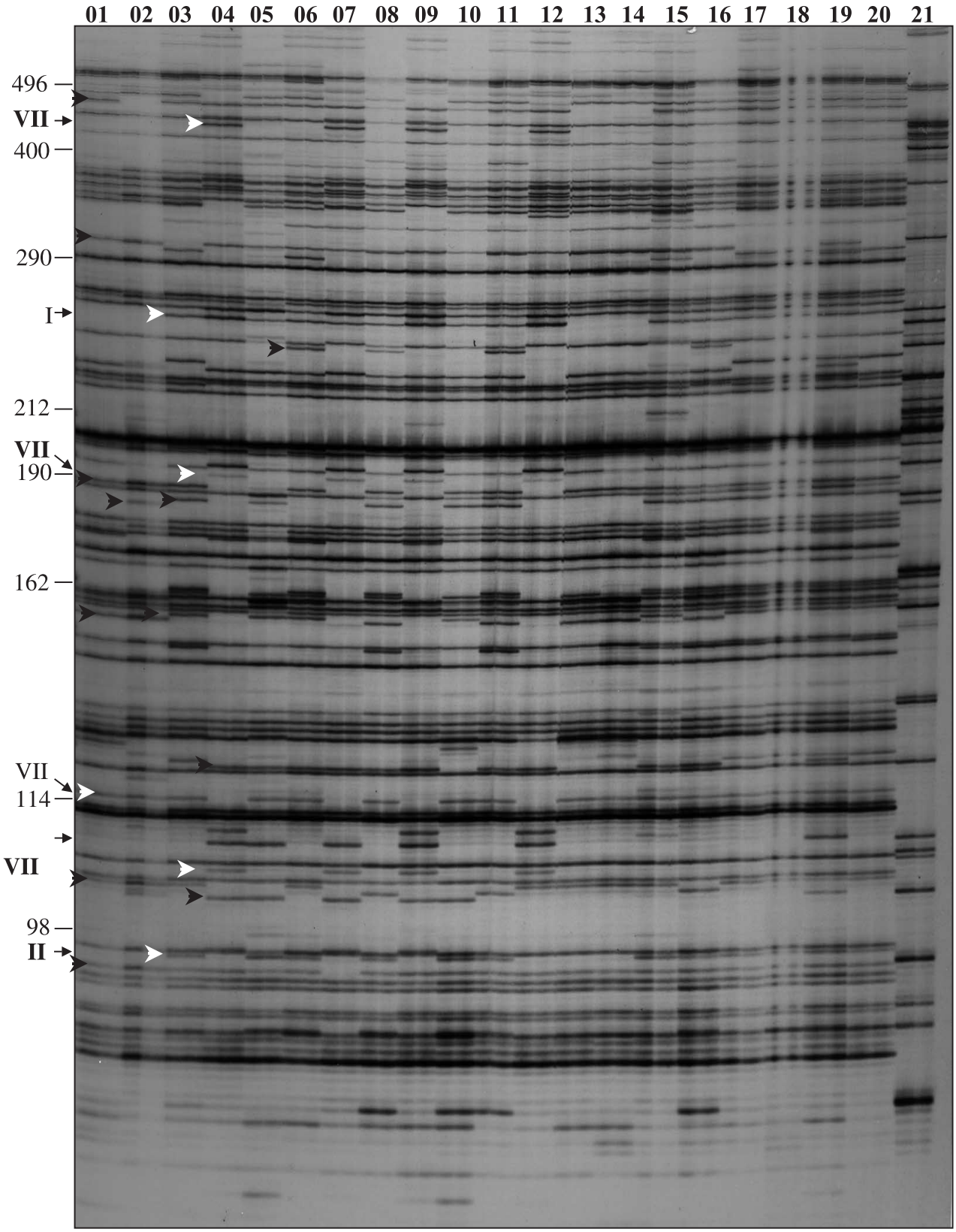


Table 2. Primer combinations for pre- and selective amplification and total and polymorphic bands scored in *Trichloris crinita* AFLP profiling.

Primers combinations[a]	Total bands	Polymorphic bands	Percentage of polymorphism (%)
mACA–eCAT	113	87	77
mACC–eCCA	87	23	34
mACC–eCTC	92	60	65
mCTC–eACA	60	19	32
mACC–eCAA	113	47	42
mCAA–eACA	90	37	41
mACC–eCGG	27	7	26
mCAA–eAAG	51	17	31
mACC–eCCA	75	34	45
mACC–eCAT	89	57	64
mCCT–eAAG	80	29	36
mCTT–eATG	50	25	50
mCCA–eAAG	112	50	45
mCAT–eAAG	110	60	56
mCGT–eAAG	68	42	61
mCGC–eAAG	48	30	62
mCCT–eACG	80	31	39
mCGC–eACG	85	43	51
mCAA–eTCG	83	29	34
mCAT–eACG	68	38	55
mAGC–eCAA	102	53	51
mAGC–eCTA	79	54	68
mACA–eCTC	52	31	60
mACA–eCTG	42	24	57
Average	77.24	39.17	49.13
Total	1855	930	

[a]e, pre-amplification primer of *Eco*RI (GACTGCGTACCAATTC); m, pre-amplification primer of *Mse*I (GATGAGTCCTGAGTAA).

were clustered according to their biomass production. Group I clustered taxa with low and medium biomass production, whereas group VII included only varieties with low yield. Group II clustered only varieties with high and medium yield and, with the exception of variety 19 (group V), included all the productive varieties.

Distinctive markers for the major clusters I, II, and VII were identified. Bands that were present, exclusively, in all the varieties of a cluster, were found for clusters I and VII (Fig. 3). Fragments present in all members of group II and absent in the rest of the varieties were not found. However, some marker bands were present in all the varieties from this group but present only in a few varieties from other groups (Fig. 3). More than 80% of the cluster-specific markers corresponded to group VII. This likely reflects the close relatedness found among the varieties in this group (Jaccard similarities ranged from 0.78 to 0.92), which was clearly separated from the other *Trichloris* taxa (Fig. 4).

Morphological and quantitative characters

Morphological and quantitative agronomic characters for the 20 *T. crinita* varieties are summarized in Table 1. Significant variation ($p \leq 0.01$) was found for all 16 traits evaluated (data not shown). Remarkable variation exists for dry matter, foliage height, culm number, and leaf blade length and width, showing at least a 2-fold increment between the lowest and highest value. The presence and density of trichomes in leaf blades (TLB) and sheaths (TLS) were also highly variable, ranging from hairless to very dense hairiness. Variety 11 had the highest values for 4 of the 7 quantitative traits (Table 1).

Euclidian distances calculated from the standardized morphological and quantitative variables, indicated that varieties 15 and 18 were most similar, whereas varieties 11 and 12 were the most distantly related (data not shown). UPGMA cluster analysis generated a dendrogram (Fig. 5) with a good fit to the Euclidian distance matrix ($r = 0.84$). Four groups were visible by cutting the dendrogram at a distance value of 4.00. The varieties were clustered according to their biomass productivity. Group I consisted of 12 varieties and included all the varieties with high and medium biomass production. No varieties with low biomass clustered in this group. Groups II, III, and IV consisted of 2, 4, and 2 varieties, respectively, and included only varieties with low productivity.

The clear separation of varieties according to their biomass production suggests that some of the traits evaluated must be associated with dry matter production. Correlation analysis demonstrated significant positive correlation between biomass production and 7 morphological and quantitative characters (Table 4). Of these, culm height, foliage height, basal diameter, and number of culms were associated with biomass production at r values higher than 0.70 (Table 4). High positive correlation was also observed among the former 4 variables and also between aerial tillers and LBW, and between LBW and LBL (Table 4).

Comparison of dendrograms based on AFLP and morphological and quantitative data

Comparison between relations depicted by the AFLP and the morphological and quantitative dendrograms showed a loose correspondence. However, partial agreements were recognized between the topologies of both dendrograms. In general, varieties were clustered according to their biomass productivity in both cases, although separation in the AFLP-based dendrogram is not as clear as in the dendrogram based on morphological or quantitative data. Group II in the AFLP cluster analysis includes 8 of the 12 varieties present in group I of the morphological and quantitative tree and both clusters include, exclusively, taxa with high and medium biomass production. Varieties 2 and 14 and varieties 4 and 9 appear closely related in both dendrograms.

Mantel Z statistics indicated a significant correlation ($r = -0.43$, $P = 0.002$) between both dendrograms (only the taxa common to both studies were considered). The negative sign is the result of comparing (Euclidian) distance and (Jaccard) similarity values.

Discussion

Analysis of biomass yield demonstrated a 9-fold variation between the most- and the least-productive varieties evaluated (Table 1). The use of productive varieties in revegetation programs will have a positive impact on range grazing and for controlling erosion. Overgrazing and erosion are the main factors contributing to the desertification of the Monte (Guevara et al. 2001; Bertiller et al. 2002).

The yield differences observed between Catamarca and

Table 3. Presence (+) or absence (–) of selected AFLP markers associated with biomass productivity in high- and low-yield accessions.

		Accessions with high biomass yield (>150 g DM/plant, >110 g DM/plant)[a]							Accessions with low biomass yield (<75 g DM/plant)						
Marker[b]	Correlation (r)[c]	**11**	**16**	**6**	19	20	10	3	12	2	7	4	9	5	14
>110 g DM/plant															
ATCC66	1.00	+	+	+	+	+	+	+	–	–	–	–	–	–	–
ATAG72	1.00	+	+	+	+	+	+	+	–	–	–	–	–	–	–
ATCG13	1.00	+	+	+	+	+	+	+	–	–	–	–	–	–	–
ATCG21	1.00	+	+	+	+	+	+	+	–	–	–	–	–	–	–
ATCC19	0.87	+	+	+	+	+	–	+	–	–	–	–	–	–	–
CAAG67	0.87	+	+	+	+	+	–	+	–	–	–	–	–	–	–
GTAG28	0.87	+	+	+	+	+	+	+	–	–	+	–	–	–	–
ATCG24	0.87	+	+	+	+	+	+	+	–	–	–	–	–	+	–
GTAG8	0.75	+	+	–	+	+	–	+	–	–	–	–	–	–	–
>150 g DM/plant															
AACC90	1.00	+	+	+	–	–	–	–	–	–	–	–	–	–	–
AACA35	1.00	+	+	+	–	–	–	–	–	–	–	–	–	–	–
CAAG44	1.00	+	+	+	–	–	–	–	–	–	–	–	–	–	–
CTCG53	1.00	+	+	+	–	–	–	–	–	–	–	–	–	–	–
ATCG29	0.87	+	+	+	–	–	–	+	–	–	–	–	–	–	–
AACA61	0.76	+	+	–	–	–	–	–	–	–	–	–	–	–	–
AACA63	0.76	+	+	–	–	–	–	–	–	–	–	–	–	–	–
CAAG17	0.76	+	+	–	–	–	–	–	–	–	–	–	–	–	–
CATG26	0.76	+	+	–	–	–	–	–	–	–	–	–	–	–	–

Note: Accessions are in decreasing order (from left to right) according to their biomass production.

[a]Two criteria for delimiting the "high biomass" class were considered: (*a*) the 1/3 most-productive varieties (> 110 g DM/plant, including accessions 11, 16, 6, 20, 10, and 3), and (*b*) more strictly, the most productive 15th percentile (>150 g DM/plant, including accessions 11, 16, and 6, indicated with bold font).

[b]AFLP markers were named with the last 2 selective nucleotides of *Eco*RI primers and the last 2 selective nucleotides of *Mse*I primers, followed by a number reflecting its relative position on the autoradiogram (bands were labeled with consecutive numbers, starting from top of the autoradiogram).

[c]All r values were significant ($p < 0.05$).

La Rioja, cannot be attributed to differences in climatic conditions, since both locations have similar mean day temperatures and receive comparable amounts of rainfall. Instead, they could be attributed to differences in soil and topographic characteristics, as these can affect the water availability of the plant.

Molecular marker analysis demonstrated that AFLP is a powerful technique for detecting DNA polymorphisms within *T. crinita*. Compared with other forage grasses, the level of intra-specific AFLP polymorphism detected here (~ 50%) is lower than reported for *Cynodon dactylon* (75%) (Wu et al. 2004) and *Poa arachnifera* Torr. (64%) (Renganayaki et al. 2001), comparable with *Uniola paniculata* (59%) (Prasanta et al. 2005), and higher than the level obtained for *Bromus catharticus* (6%) (Puecher et al. 2001).

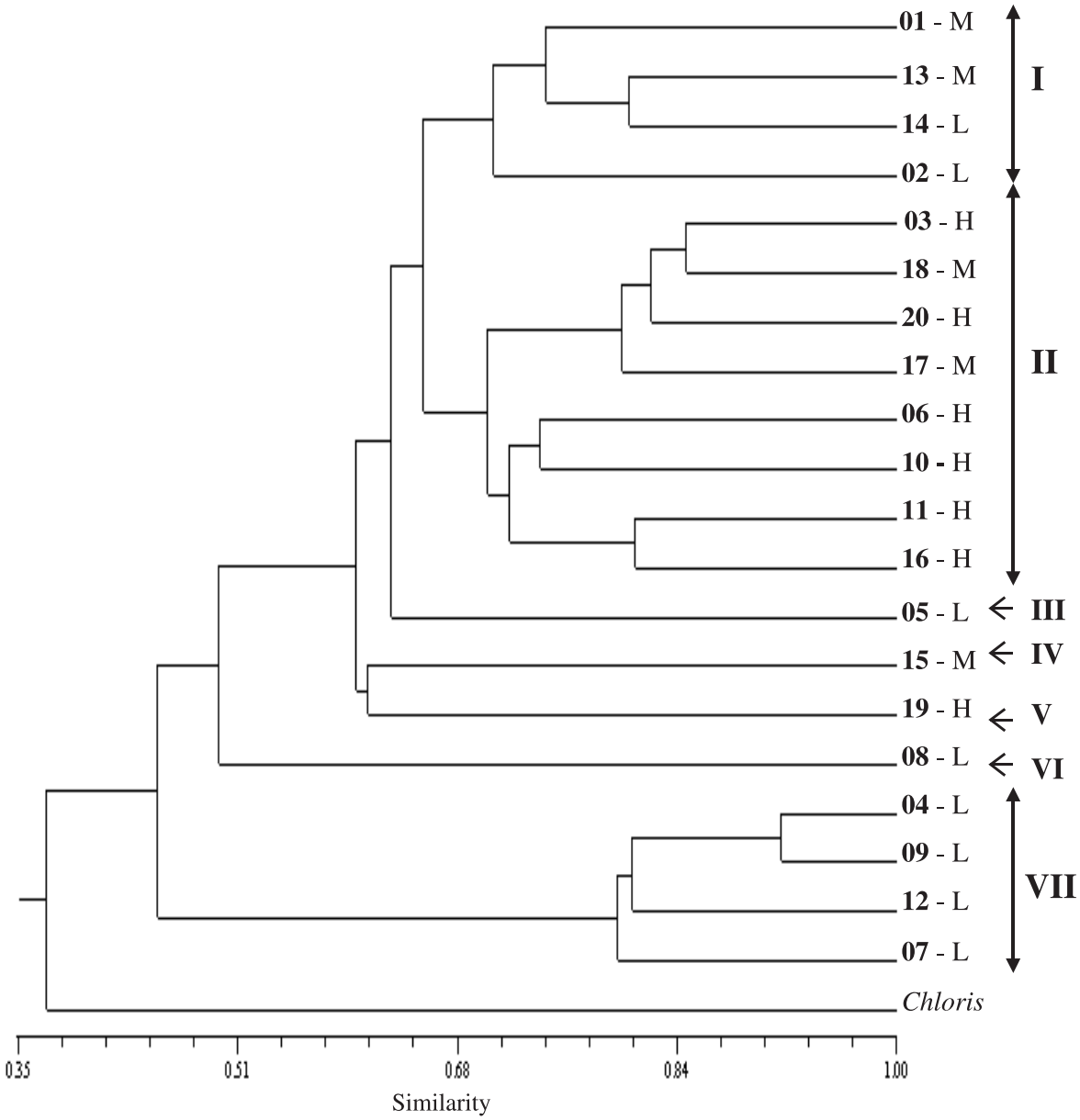
Distinctive AFLP profiles allowed the genotyping of all *T. crinita* varieties. Two or 3 selected primer combinations were, in most cases, enough to discriminate among all the samples. However, it is unlikely that a few randomly chosen primers will be similarly efficient in discriminating *T. crinita*, as suggested by the extensive primer-to-primer variation for yielding AFLP polymorphisms (Table 2).

Biomass yield is one of the most important traits of any forage species. We identified markers for high biomass production in *T. crinita*. Particularly useful are 8 markers that showed complete correlation ($r = 1.00$) with this trait. Such markers can be used to assist selection for high forage productivity. Moreover, their usefulness would be greatly expanded if they could be cloned and the sequence used to develop more robust PCR-based markers (Paran and Michelmore 1993).

AFLP-based cluster analysis related the varieties in general accordance to their biomass production. This separation is more evident for the high- and low-biomass materials. For example, all of the productive varieties were clustered separately from the low-yield varieties and one major cluster (VII) included only samples with low biomass (Fig. 4). The medium-yield biomass varieties grouped together with low- or high-yield varieties (clusters I and II) indicating a significant amount of shared markers between some materials of the intermediate class and materials from both the high and low biomass categories.

Clustering of varieties was not associated with geographic distance or habitat conditions. For example, plants collected in the extremely dry habitat of Encón (mean annual rainfall is 104 mm), included low-, medium- and high-biomass varieties that clustered over the 3 major AFLP groups. The fact that different genotypes and morphotypes coexist in natural communities suggests that *T. crinita* populations are genetically heterogeneous. The lack of natural barriers probably facilitated the spread and mixture of genetic materials among populations. Granivorous birds, small mammals, and ants play an important role in seed removal and dispersal in Monte (Lopez de Casenave et al. 1998). Another explanation for the lack of geographical association is the possible low representativeness of the original populations by the selected

Fig. 4. Phenetic relations among 20 *Trichloris crinita* accessions from UPGMA cluster analysis of the Jaccard similarity coefficient matrix generated using data from 930 polymorphic AFLP bands obtained with 24 primer combinations. Roman numbers indicate groups discriminated at 65% similarity. An accession from *Chloris virgatum* (*Chloris*) was included in the analysis as a putative outgroup. Taxa are indicated by the accession number, followed by a letter indicating whether they have high (H, > 110 g DM/plant), medium (M, from 75 to 100 g DM/plant), or low (L, fewer than 75 g DM/plant) biomass yield.



plants (some could have been a rare event in the population), as they were selected only on the basis of morphological differences.

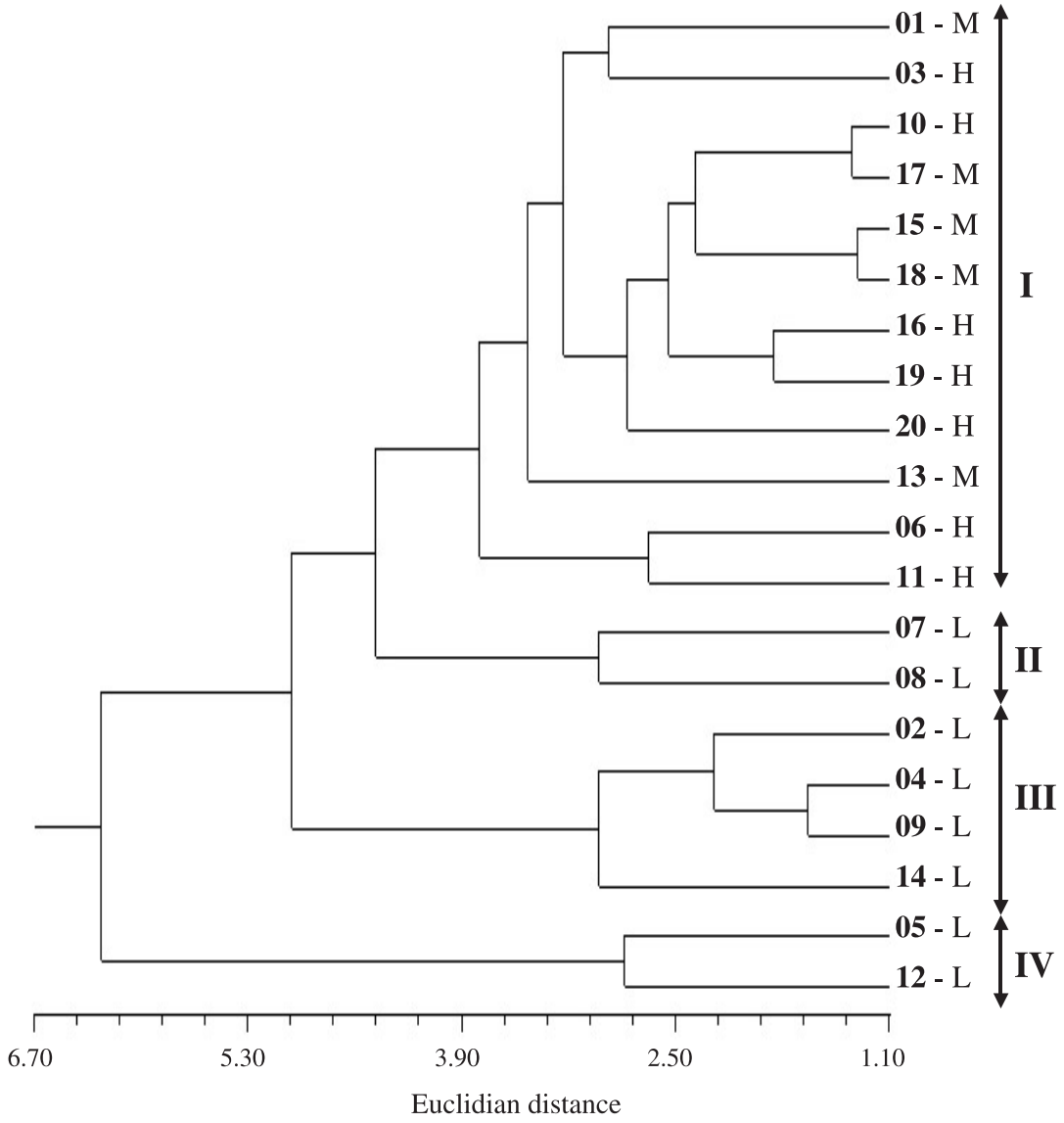
We found distinctive AFLP bands for the major clusters I, II, and VII. These markers can be used to rapidly screen plants of *T. crinita* and determine whether they are associated with a certain cluster of genotypes. Because clustering based on AFLPs was associated with biomass production, these markers also represent an indirect way of screening for biomass yield.

Cluster analysis based on morphological and quantitative agronomic data revealed a clear separation of varieties according to their biomass production. A single cluster (I) grouped all the medium- and high-yield varieties, while all the low-yield varieties were distributed in 3 other clusters (II, III and IV). This suggests that morphometric characters can be useful for delimiting varietal groups according to their biomass yield.

Correlation analysis among the variables demonstrated that varieties with high biomass tend to have tall foliage and a large number of long culms holding broad-blade leaves. Also, a large basal diameter and the presence of aerial tillers and of trichomes on the edge of the leaf sheath (TELS) were associated with productive phenotypes. Correlated traits that are easy to visualize and score can be used for rapid selection of productive phenotypes in natural *T. crinita* populations.

Morphological characters are advantageous over AFLPs owing to their simplicity; however, frequently they cannot

Fig. 5. Dendrogram for 20 *T. crinita* accessions obtained from UPGMA cluster analysis of Euclidian distance based on morphological and quantitative agronomic characters. Taxa are indicated by the accession number, followed by a letter indicating whether they have high (H, > 110 g DM/plant), medium (M, from 75 to 100 g DM/plant), or low (L, fewer than 75 g DM/plalnt) biomass yield. Roman numerals indicate groups of accessions with Euclidian distances smaller than 4.0.



discriminate between closely related plants. Both types of markers can complement each other for an efficient selection process. For example, morphological traits can be used in rapid pre-screenings to subdivide materials into groups (e.g., plants with and without TELS), which can be evaluated in more detail using AFLP markers.

The characterization of these materials at different levels (AFLPs, morphology and quantitative agronomic traits) will enhance the use *T. crinita* for specific purposes. For example, stabilized varieties varying in several traits of agronomic interest (e.g., biomass yield and growth habit) are now available to be used in range seeding for either livestock grazing or prevention of land degradation. From a genetic and breeding point of view, if the inability of these materials to intercross could be somehow circumvented, a number of important studies would be possible. The construction of linkage maps, QTLs analysis (e.g., for biomass, nutritional content, palatability), and the acquisition — through sexual reproduction — of phenotypes that combine specific traits of interest, are just a few examples. In all of these studies, prior knowledge about genotype relationships and their variation for a given trait of interest is crucial for obtaining the desired results. For this reason, the 20 *T. crinita* varieties and the data presented herein represent the starting point of future genetic studies in this forage grass.

Chloris virgatum, the species used as a putative out-group in our AFLP analysis, is a close relative of *Trichloris* and the 2 genera share many morphological features. This has lead to controversies of whether *Chloris* and *Trichloris* are the same genus (Anderson 1974) or whether they should be regarded as different genera (Nicora and Rúgolo de Agrasar 1987). Our results from the AFLP cluster analysis, demonstrating a clear separation of *Chloris virgatum* from all the *Trichloris* varieties, support the separation of *Chloris* and

Table 4. Correlation values (r) among 16 morphological and quantitative agronomic traits.

	DM	CH	FH	BD	CN	TLS	TELS	TLB	CS	CINo	CNo	CL	AT	GH	LBW
CH	0.75**														
FH	0.89**	0.85**													
BD	0.83**	0.81**	0.89**												
CN	0.74**	0.68**	0.77**	0.67**											
TLS	–0.18	–0.51*	–0.39	–0.46*	–0.4										
TELS	0.53*	0.37	0.49*	0.21	0.16	0.18									
TLB	–0.33	–0.53*	–0.45*	–0.49*	–0.59**	0.58**	0.3								
CS	0.21	0.21	0.24	0.25	0.32	0.11	0	–0.2							
CINo	–0.1	0.05	0	–0.18	0.28	–0.12	–0.18	–0.2	0.45*						
CNo	0.39	0.47*	0.47*	0.41	0.22	–0.29	0.28	–0.4	0.18	0.19					
CL	0.43	0.33	0.41	0.33	0.13	0	0.57*	0.17	0.26	0.12	0.66**				
AT	0.55*	0.58	0.65**	0.51	0.33	–0.2	0.57*	0	0.39	0.17	0.68**	0.66**			
GH	0.28	0.1	0.24	–0.1	0.26	0.27	0.57*	0.21	0.1	0.26	–0.1	0.14	0.29		
LBW	0.55*	0.49*	0.56*	0.37	0.32	0.16	0.63**	0.17	0.26	0.17	0.38	0.45*	0.80**	0.4	
LBL	0.27	0.42	0.4	0.22	0.03	0.16	0.60**	0.24	0	–0.2	0.13	0.37	0.51*	0.3	0.73**

Note: AT, aerial tillers; BD, basal diameter; CH, culm height; CINo, color of internode; CL, color of ligule; CN, culm number; CNo, color of node; CS, color of spike; DM, dry matter; FH, foliage height; LBL, leaf blade length; TELS, trichomes on the side of leaf sheath; TLB, trichomes in leaf blade; TLS, trichomes on leaf sheath. Correlation values above 0.7 are indicated in bolded letters.
*, significance at $p \leq 0.05$; **, significant at $p \leq 0.01$.

Trichloris as 2 distinct genera. Further analysis including a larger number of *Chloris* species should provide a more definite answer to this issue.

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