Journal of Agricultural Science, *Cambridge* (2000), **134**, 207–212. *Printed in the United Kingdom*

Estimation of repeatability and phenotypic correlations in *Eragrostis curvula*

M. A. DI RENZO[1*], M. A. IBAÑEZ[1], N. C. BONAMICO[1] AND M. M. POVERENE[2]

[1] *Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina*
[2] *Departamento de Agronomía, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina*

(*Revised MS received 14 September 1999*)

SUMMARY

Eragrostis curvula (Schrad.) Nees *s. lat*., a highly polymorphic polyploid complex, can be considered as one of the most important warm season perennial grasses for the semi-arid regions of central Argentina. In apomictically propagated and perennial plants such as weeping lovegrass, where successive measurements can be done across time, repeatability estimates provide an indication of the degree of influence of permanent effects on the phenotypic variation and allow prediction of future performance from past records. Analysis of variance of the experiment showed highly significant variation ($P < 0{\cdot}01$) for the main factors in all four traits. Although there was very high variability between cuts, hybrids of *E. curvula* exhibited considerable inter-entry variability, in particular for those traits determining forage yield. Repeatability calculated in this experiment was highest for the crown diameter (0·86), leaf length (0·84) and dry matter (0·84), while in panicle number (0·66) it was lowest. Repeatability estimates for the vegetative characteristics indicate small effects of temporal environment. The four traits studied, including panicle number with their moderate repeatability, do not require an essentially different number of observations to obtain measures at the same level of accuracy. For vegetative characters two harvests provided 98% of the accuracy of the total obtained with four cuts, and for panicle number the same percentage was obtained for three harvests. This stability of performance is a desirable characteristic for grass cultivars. Patterns of trait associations were also described. Because leaf length is closely associated with dry matter and has high repeatability, to use leaf length as an indirect evaluation criterion should be almost as efficient as direct evaluation for aerial biomass yield. Reliable estimates of parameters such as repeatability and phenotypic correlation are needed for prediction of production values and for the design of efficient improvement programmes. For genotype evaluation additional research is required to quantify the extent of genotype × environment interaction across years and localities of semi-arid regions.

INTRODUCTION

Weeping lovegrass, *Eragrostis curvula* (Schrad.) Nees *s. lat*., a native of southern Africa, has been introduced for soil conservation purposes in Argentina from the USA and has naturally spread due to its high adaptability to marginal environments. Because several ecotypes of this species have been collected and released, most lovegrass cultivars have an undefined origin (Medina *et al*. 1985; Di Renzo *et al*. 1992). *E. curvula*, a highly polymorphic polyploid complex (Poverene & Curvetto 1989; Poverene & Voigt 1997), can be considered as one of the most important warm season perennial grasses for the central semi-arid regions of Argentina.

Sexual and facultative apomictic plants (Voigt 1971; Voigt & Bashaw 1972, 1976) allow recombination of desirable agronomic characteristics from different strains and are the basis of hybridization breeding schemes for genetic improvement of this species (Voigt 1984). Due to the restricted germplasm used in Argentina and the need to find and incorporate alternative varieties in sustainable systems, cooperative USA–Argentina evaluation studies of *E. curvula* were conducted. Seed dormancy in lovegrass hybrids was studied in cooperation for establishment of plots in the northern and southern hemispheres (Voigt *et al*. 1996). The present work focuses on a subset of hybrids obtained in Temple (USA) to assess the extent of phenotypic variation for yield and other evaluated agronomic traits. Patterns of trait association at the phenotypic level and repeatability of each trait are described and added to other studies, such as

* To whom all correspondence should be addressed. Email: mdirenzo@ayv.unrc.adu.ar

quality of forage (Voigt 1984) and stability of the germination (Ibañez *et al.* 1998). There is no available information regarding the repeatability and the relationship between yield and other agronomic trait measurements among lovegrass cultivars.

Repeatability, an estimation of the strength of the relationships between repeated phenotypic values for a trait measured on the same individual, is related to broad sense heritability of characters among a group of fixed entities such as clones (Simmonds 1979; Dias & Kageyama 1998). Estimation of repeatability involves the partitioning of phenotypic variance into components such as permanent effects and temporary environmental effects (Falconer & Mackay 1996). The permanent influences, which include the genetic and general environmental variation, assess the between genotype variances. The temporary environmental effect reflects the between multiple measurement variation on the same genotype. Because estimates of permanent and temporary effects and repeatability coefficients for agronomical attributes are functions of variance, these parameters may be specific for a particular population, environmental conditions and time period. In apomictically propagated and perennial plants such as lovegrass, where successive measurements can be done across time, repeatability estimates provide an indication of the degree of influence of permanent effects on the phenotypic variation and allow the prediction of future performance from past records (Falconer & Mackay 1996).

The specific objectives of this study were (i) to estimate variances and repeatability for dry matter, leaf length, crown diameter and panicle number, (ii) to calculate gain in accuracy from multiple measurements and (iii) to determine the phenotypic correlations among traits. These estimations, which may contribute to the design of efficient enhancement programmes and evaluation activity, and which may serve to formulate some recommendations regarding the tested lovegrass cultivars, could be very helpful in subsequent predictions of the value of the genotypes under selection, especially for the improvement of yield, which is a complex character.

MATERIALS AND METHODS

Strain and observations

The experimental field was located at the Universidad Nacional de Río Cuarto (33°8′S, 64°20′W; 334 m asl). The trial was established in March 1996 in a Typic Hapludulls soil. The vegetative traits, aerial biomass yield determined by dry matter weight (DM), leaf length (LL) and crown diameter (CD) and the reproductive trait, panicle number (PN) were measured in four cuts. Forage was harvested at a height of 8 to 10 cm during the 1997/98 growing season. The herbage mass harvested was dried in a forced air oven at 70 °C for 48 h for dry matter weight (g dry weight/plant) determination. Prior to harvest panicle number (number of panicles per plant) and leaf length (cm) were measured. Leaf length was recorded as the distance from the ground surface to the tip of a sample of fully extended leaves. After the harvest, crown diameter (cm) was registered as the means of two perpendicular readings.

The experiment comprised a random group of 18 hybrids, which are part of the germplasm proposed for evaluation experiments conducted in cooperation (Table 1). The hybrids involved five different sexual, tetraploid hybrids as female parents and nine apomictic selections as male parents, obtained by Dr P. W. Voigt at Temple, Texas, USA.

Forage was harvested at the beginning of November, when most entries start reproductive growth. Three regrowths were harvested at arbitrarily fixed intervals of 30–40 days. After each harvest the plot was fertilized with 100 kg N/ha as urea. Entries were germinated in a greenhouse and seedlings were transplanted to a trial field in a randomized complete-block design with three replications. All plants were planted with a spacing of 1 m between rows and 0·3 m between plants. A plot consisted of a single row of 12 plants. Evaluation as individual spaced plants minimizes seed requirements and improve the performance in grass pastures. The experiment was surrounded by a guard row of Tanganyika plants, a variety locally well adapted, and the experimental area was maintained in a weed-free condition by mechanical cultivation and hand-weeding. Plot sampling was made on a per-plant basis with only those plants that were fully competitive in order to keep the same level of competition effects between plants.

Statistical analysis

A two-way analysis of variance (ANOVA), was performed on the yield and on the related attributes averaged per entry per cut, using Statistical Analysis System (SAS), General Linear Models (GLM) procedure (SAS 1990). To homogenize the variability among the treatments logarithmic $(y+1)$ transformations on all attributes were done, except on the panicle number which was transformed to square root $(y+0{\cdot}5)$ before performing ANOVA. The following statistical model was used to estimate the variances of cutting-entry data:

$$y_{ij}=\mu+g_i+c_j+(gc)_{ij}+\epsilon_{ij}$$

In this expression, the y_{ij} is the observation of the ith entry g, jth cut c; μ is the overall mean, gc is the interaction, and ϵ is the residual error. In the model all effects were assumed as random. Variance components were estimated by equating mean squares to their expectations and solving for the component.

Table 1. *Means of each trait averaged over four successive harvests for the 18 hybrids of lovegrass*

Entry	Code	Dry matter	Leaf length	Crown diameter	Panicle number
122-21-83	1	72·15	54·75	29·24	2·16
102-93-84	2	50·89	57·81	29·61	1·89
103-69-84	3	50·11	50·85	28·54	1·26
104-82-84	4	55·33	48·73	30·44	2·79
108-20-84	5	83·92	65·96	32·15	1·76
110-92-84	6	58·97	53·96	31·18	1·47
110-49-84	7	66·65	59·17	30·03	1·46
111-27-84	8	67·87	56·42	30·89	2·10
111-28-84	9	46·51	50·88	28·78	2·09
117-93-84	10	49·21	49·24	28·11	3·53
119-67-84a	11	55·54	57·83	27·72	2·57
119-67-84b	12	39·01	55·56	24·82	2·19
124-11-84	13	63·55	54·79	27·58	1·25
126-92-84	14	67·06	50·40	30·97	0·90
128-2-84	15	68·78	64·25	29·34	1·67
129-85-84	16	32·32	45·92	25·51	2·68
129-95-84	17	68·76	49·40	34·10	3·14
101-85-84	18	50·50	55·23	29·54	2·20

Their standard errors were estimated using conventional procedures (Anderson & Bancroft 1952).

Repeatability for dry matter, leaf length, crown diameter and panicle number was calculated from component of variance applying the equation:

$$R = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$$

where the component σ_g^2 represents the between-entry variance and estimates genetic and general environmental effects; the component σ_e^2 – the residual variance – is the within-entry variance and estimates temporary environmental effects. The procedures of Becker (1984) were used to estimate standard error for repeatability:

$$\text{S.E.} = \sqrt{\{2\ (1-R)^2\ [1+(c-1)R]^2/c(c-1)\ (g-1)\}}$$

where c and g refers to the number of cuts and genotypes respectively. When the repeatability is close to 1, error measurement is negligible against inter-entry variability. A weak repeatability means that measurement variability within the genotype is more important than between genotype variability. On the other hand, repeatability of each character, as an intraclass correlation coefficient, gives a measure of intrinsic variability allowing comparisons to be made between measurements expressed in different units. From multiple measurements, gain in accuracy (Bourdon 1997) was calculated as:

$$r_{\text{AC}} = \sqrt{\{(cR)/[1+R(c-1)]\}}$$

For each entry, phenotypic associations between the four *Eragrostis* traits considered were estimated as Pearson product-moment correlations to determine trends in the data.

RESULTS

The means for dry matter, leaf length, crown diameter and panicle number of the eighteen entries averaged over the four cuts are presented in Table 1. Entry 5 showed the highest mean values for dry matter and leaf length and occupied the second place for the crown diameter. Entry 17 occupied the first place for the crown diameter and entries 10 and 17 occupied the first and second place respectively for panicle number. Genotypes showed consistent trend over the four successive harvests, especially for dry matter. This character increased from the first to the third cut and decreased in the fourth cut for all entries, except for 16, 9, 8 and 11. The magnitude of variation among entries for this trait, remained relatively constant among cuts although important differences among entries were found in the second and third harvest.

Analysis of variance of the experiment indicated highly significant variation ($P < 0{\cdot}01$) for the main factors in all four traits. Although the variability between cuts was very high (Table 2), variability among entries showed substantial differences between genotypes. Non-significant genotype × cut interactions were observed in all characters except panicle number.

Estimating repeatability and accuracy

Repeatability calculated in this experiment was highest for the crown diameter (0·86), leaf length (0·84) and dry matter (0·84), while in panicle number (0·66) it was the lowest. Because the vegetative

Table 2. *Analysis of variance for four traits of 18 entries of lovegrass across four cuts. Variance component and repeatability estimates with their corresponding standard errors* ($\pm$ S.E.)

Source of variation	D.F.	Mean square DM	LL	CD	PN
Entry (E)	17	0·60**	0·10**	0·07**	5·76**
Cut (C)	3	9·31**	2·60**	0·30**	32·50**
E × C	51	0·06	0·01	0·01	2·97**
Error	142	0·08	0·01	0·01	1·10
σ_g^2		0·39 ($\pm$0·016)	0·07 ($\pm$0·003)	0·05 ($\pm$0·002)	2·09 ($\pm$0·156)
σ_c^2		0·08 ($\pm$0·008)	0·01 ($\pm$0·001)	0·01 ($\pm$0·001)	1·10 ($\pm$0·161)
R		0·84 ($\pm$0·053)	0·84 ($\pm$0·052)	0·86 ($\pm$0·046)	0·66 ($\pm$0·096)

*, **, The F value in the variance analysis is significant at the 0·05 and 0·01 levels of probability, respectively; σ_g^2 and σ_c^2 are estimates of the corresponding components of variance in an among- and within-classes analysis.

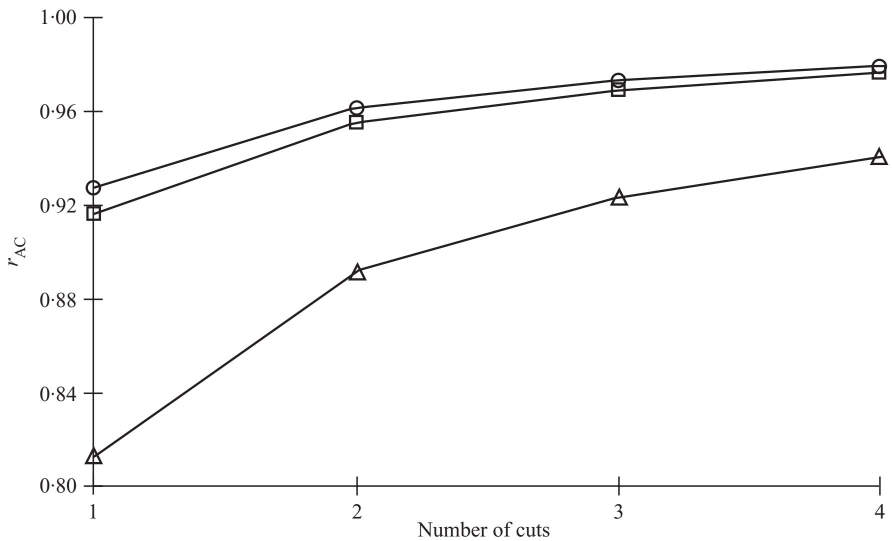

Fig. 1. Gain in accuracy (r_{AC}) when the number of harvests increases from one to four in dry matter and leaf length (○), crown diameter (□) and panicle number (△) traits.

characters measured show a non-significant genotype × cut interaction, temporary environmental effects were estimated by pooling the mean squares for interaction and residual effects.

Figure 1 shows the gain in accuracy expected from multiple measurements when the number of harvests increases from one to four. Two cuts result in an increase in accuracy over a single measurement of 4% for dry matter and leaf length, 3% for crown diameter and 8% for panicle number. Three cuts result in a further increase in accuracy of 3 and 5% for vegetative characters and 11% for panicle number. For dry matter, leaf length and crown diameter two harvests provided 98% of the accuracy of the maximum obtainable with four cuts. For panicle number a similar percentage was obtained for three harvests.

Phenotypic correlations

Pearson correlations between dry matter and leaf length indicate that there are no negative associations (Table 3). Dry matter was significantly correlated with leaf length except for entry 16 ($r = 0·55$; $P > 0·05$). The DM-LL correlations ranged between 0·95 (entry 11) and 0·55 (entry 16) with a mean of 0·81. Phenotypic correlations for all other trait combinations were generally low and non-significant, although panicle

Table 3. *Correlation coefficients between four agronomic traits for each entry of lovegrass*

Entry	DM-LL	DM-CD	CD-LL	DM-PN	LL-PN	CD-PN
1	0·93**	0·41	0·45	0·01	−0·31	0·06
2	0·91**	0·10	0·13	0·00	−0·24	−0·39
3	0·75**	−0·23	0·28	0·15	−0·12	−0·09
4	0·77**	0·58*	0·37	0·42	0·15	0·13
5	0·84**	−0·09	−0·03	0·11	−0·07	−0·25
6	0·79**	−0·09	0·27	0·13	−0·32	−0·11
7	0·94**	0·31	0·40	−0·09	−0·31	−0·76**
8	0·76**	0·27	0·51	0·33	−0·22	−0·10
9	0·73**	0·31	0·32	0·36	−0·10	−0·43
10	0·66*	0·56*	0·24	−0·11	−0·58*	0·25
11	0·95**	0·06	0·19	−0·20	−0·29	−0·29
12	0·60*	0·13	0·26	0·29	−0·22	−0·26
13	0·85**	0·32	0·51	0·21	−0·10	−0·07
14	0·93**	0·22	0·49	−0·25	−0·41	−0·54
15	0·93**	0·05	0·06	−0·11	−0·24	−0·31
16	0·55	−0·07	0·27	0·67*	0·27	−0·30
17	0·90**	0·23	0·35	−0·40	−0·53	−0·24
18	0·74**	0·34	0·07	0·19	−0·22	0·05

*, ** Correlation significantly different from zero at the 0·05 and 0·01 levels of probability, respectively.

number was negatively correlated with leaf length and crown diameter.

DISCUSSION

During the growing season considered, the entries showed consistent trends over the four successive cuts, indicating a relatively high level of genotype consistency. The extent to which the differences are retained over a range of environments will be examined in future studies. Observations repeated in the course of time allow an estimation of repeatability and indicate stability which reflects genotype × environment interaction effects (Bos & Caligari 1995). The high repeatability obtained for dry matter, leaf length, and crown diameter could be explained by apomictic reproduction as well as by the absence of genotype × cut interactions in these characters. This stability in performance is a desirable characteristic for grass cultivars. This result contrasts with previous studies (Ibañez *et al.* 1998), conducted to infer the implantation capacity and evaluate the initial growth of different lovegrass cultivars, where genotype × environment interaction was highly significant. Dissimilar results of repeatabilities have been reported in quackgrass (*Elymus repens* L.) (Casler *et al.* 1998), alfalfa (*Medicago sativa* L.) (Scossiroli *et al.* 1963) and white clover (*Trifolium repens* L.) (Jahufer *et al.* 1997) for traits related to forage yield. Repeatability estimates for these vegetative characteristics at each cut indicate small effects of temporal environment, in comparison with permanent effects and suggest that an increase in the number of measurements does not reduce the amount of variance due to temporal environment. In addition, the four traits studied, including panicle number with their moderate repeatability, do not require an essentially different number of observations to obtain measures at the same level of accuracy. Estimates of repeatability, which were reliable as reflected by their small standard errors, and the small gains in accuracy obtained with multiple measurements, indicate that two cuts are sufficient to evaluate this germplasm. Studies on the repeatability of traits are specific to genotypes and to environments. The repeatabilities presented here, which were calculated from spaced plants, i.e. at a very low plant density, may differ from those assessments conducted with plants grown in standard, dense sward conditions, although low densities are commonly adopted for genotype evaluation. When trials are visually less uniform due to management, stress or severe drought, measuring three successive harvests may be preferred in order to estimate differences.

Phenotypic correlations are purely environmental, because they are calculated from genetically uniform clones propagated apomictically (Simmonds 1979). Since dry matter, the agronomic character of greatest interest, and leaf length are closely associated, as expected for morphological reasons, evaluating one should also concurrently evaluate the other. Since leaf length can be readily determined, this consistent association may be a useful criterion for indirect evaluation and selection purposes. The lack of significant associations between dry matter and both crown diameter and panicle number suggest that these two traits may not be a good indirect criterion for improving evaluation of forage yield. However, the lack of negative associations between DM-CD and CD-LL, observed in the majority of entries, would not restrict the evaluation and selection of aerial biomass yield. Panicle number, which has a negative correlation with both leaf height and crown diameter probably as a consequence of their opposite relationship with maturity, may not be a useful predictor of forage yield.

The present findings, concerning the extent of phenotypic variation, repeatability and association of traits, although they apply primarily to the specific collection of material tested in cooperative USA–Argentina studies, are promising for evaluation programmes.

As a consequence of the previous discussion, it may be concluded that hybrids of *E. curvula* exhibited considerable inter-entry variability, particularly for those traits determining forage yield. This variation, related to the temporary environmental effects, determines the highest value of repeatability and as a consequence, only a few observations are required for a reliable estimation of the productive value of a genotype. Because leaf length has a high repeatability and is closely associated with dry matter, use of leaf length as an indirect evaluation criterion may be almost as efficient as direct evaluation of yield. Assessing repeatability and association between traits are of interest because they may improve the efficiency of evaluation by contributing to the accurate prediction of production values and reducing cost, effort and time required in breeding programmes (Dias & Kageyama 1998). For the selection of genotypes which could be used in sustainable systems additional research is required to quantify the extent of genotype × environment interaction across years and localities of semi-arid regions. Such further investigations could be facilitated by knowledge of the repeatability coefficients and by development of cooperative evaluation studies.

This work was supported by grants from Universidad Nacional de Río Cuarto and Consejo de Investigaciones Científicas y Técnicas de Córdoba (CONICOR), Argentina. The authors wish to express their gratitude to Dr I. Tiranti for reviewing the manuscript and to Professor I. Martínez for helping with the English translation.

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