Characterization of wild perennial ryegrass populations from Galicia (Spain)

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SUMMARY

Forty seven spontaneous populations of perennial ryegrass were collected as seeds in 1985 en Galicia (North-West Spain), and evaluated at Investigaciones Agrarias Galicia Station, near La Coruña, in a spaced plants nursery. Twelve agronomic traits were scored since 1986 to 1988.

Indigenous populations show, on average, poorer agronomic performance than control cultivars. Since they have much wider range of variation, the best ones can be as good as commercial lines for some traits. A principal component analysis has been used to summarize the data from population means and a hierarchical clustering method using Euclidean standardized distance has been performed.

This classification leads to a partition into 4 clusters, one of them grouping the most interesting populations. It is noticeable that cv «Brigantia», which was constructed by mass selection from a wild population from La Coruña, appears to be the best of the control when tested in Galicia.

A factorial discriminant analysis was then carried out using ecological (climate, soil) data from the collection sites. This analy-

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sis succeeded in founding linear combinations of ecological traits which discriminate the clusters established from agronomic traits only.

The implications for genetic resources management and genetic improvement are discussed.

Key words: **Lolium perenne**, genetic resources, hierarchical clustering, wild populations, ecological traits.

Introduction

The main aim of collecting wild populations of forage species is to have a broad genetic basis for genetic improvement programmes (Gallais, 1977, 1978, Tyler, Jones, 1982). Plant breeders should have available genetic resources which are as representative as possible of the genetic diversity of the species (Pernes, 1984).

After collection, evaluation of genetic resources provides the knowledge of the adaptation of populations to several environmental conditions (Jones, Walker, 1983).

Multivariate analysis methods have been used by many authors on perennial ryegrass (Hayward, Breese, 1968, Charmet et al., 1987, Falcinelli et al., 1987), italian ryegrass (Hayward et al., 1982) and tall fescue (Veronesi, Falcinelli, 1988). Hierarchical clustering appears to be a useful method for classifying large collections of genetic resources and setting up core collections (Peeters, Martinelli, 1989).

In a widely spread grass species like perennial ryegrass, the genetic diversity is expected to be high in relation to the diversity of ecological conditions (climate, soil, grassland management...) in their habitat. If this is the case, any relationship found between agronomic traits and ecological data from the collection site could provide a guide for further collection aimed at gathering wild material with particular characteristics (Tyler, Chorlton, 1976).

This paper reports the results of agronomic evaluation of spontaneous ryegrass populations from Galicia and the relationships between quantitative traits of agronomic interest and some ecological characteristics of the collection sites.

Material and methods

Forty-seven seed samples of spontaneous populations of perennial ryegrass *(Lolium perenne L)* were collected in 1985 in Galicia.

On each collecton site, 2 spikes per plant from at least 30 plants were sampled from a visually homogeneous area in order to represent a panmictic population (YONEZAWA, 1985, TYLER, 1987). Geographic coordinates of the collection sites are available on request.

Every populations and 4 control cultivars, namely cv «Francés», «Reveille», «Taptoe» and «Brigantia» (a local variety bred at INIA La Coruña), were sown in a greenhouse in November 1985, then trasplanted in March 1986 to the field at Mabegondo (INIA Station, near La Coruña). Mabegondo has an annual average temperature of 12.3° C (4.3° C and 21.1° C for the coldest and hottest month, respectively). The annual rainfall is 876 millimeters, the soil is a brown acid soil (pH = 4.9) with no deficiency in any particular element.

The experimental design consisted in six randomized complete blocks with 10 plants of each population in each block, 3 of them being cut frequently (every 3 weeks), the last 3 being cut every 5 weeks after a conservation cut at heading time.

The following traits were observed from 1986 (planting year) to 1988 and noted on a 1-9 visual scale, except for heading date.

In 1986:

— HAB: Growth habit from 1 = erect to 9 = prostate.

— TIL: Tillering: from 1 = few tillers to 9 = many.

— RUS: Crown rust susceptibility: from 1 = resistant to 9 = susceptible.

— AUG: Autumn growth: from 1 = weak to 9 = susceptible.

In 1987:

— ESG: Early spring growth (March) from 1 = weak to 9 = vigorous.

— SPG: Spring growth (in May): from 1 = weak to 9 = vigorous.

— SRUS: Crown rust susceptibility in May from 1 = resistant to 9 = susceptible.

— HED: Heading date: days from January 1st.

— AMH: Aftermath heading (in July) from 1 = none to 9 = many spikes.

— SUG: Summer growth (end of July): from 1 = weak to 9 = vigorous.

— AURG: Autumn regrowth (october): from 1 = weak to 9 = vigorous.

In 1988:

— PER: Persistence: number of living plants after two years.

16 ecological traits, either from each collection sites (soil sample data) or from the nearest climatic station:

— ETP: Potential evapotranspiration (mm).

— R: Annual rainfall (mm).

— AmT: Average temperature of the coldest month (°C).

— AMT: Average temperature of the hottest month (°C).

— Wdef: Water deficit from June to September (mm.).

— ASL: Altitude (m.a.s.l.)

— SAN: Sand content of the soil (percent %).

— LOA: Loam percentage.

— CLA: Clay percentage.

— OM: Organic matter content (%).

— pH: pH (measured by water technique).

— P: Phosphorus content (ppm).

— K: Potassium (meq/100 g dried soil).

— Ca: Calcium (meq/100 g dried soil).

— Mg: Magnesium (meq/100 g dried soil).

— Al: Aluminium (meq/100 g dried soil).

Although observed on a discrete scale, the distribution of most agronomic traits appears to fit a Gaussian distribution. Therefore data from individual plants were submitted to a two analysis of variance with blocks as fixed effects and populations as a random effects. It was thus possible to test the significance of between populations variation against error (which is within population variation) and to estimate adjusted population means (i.e. corrected for the unequal numbers of plants in each block when due to missing plants).

A principal component analysis using the correlation matrix between population means, with agronomic traits as active variates and ecological traits as illustrative was carried out.

The derived principal component values of each population were then used for hierarchical ascendant clustering with a reciprocal neighbour algorithm using Ward's criterium of aggregation. This method aims to maximize the between cluster variance and minimize variance within (BENZECRI et al., 1979).

The principal component analysis showed that some ecological traits were correlated with the principal components. Factorial discriminant analysis was used to investigate the association between ecological traits and the clusters obtained from agronomic data. This method uses the distance of Mahalanobis on the ecological traits.

RESULTS

The main results of the analysis of variance are presented on table 1. The population effect being always significant, the adjusted population means are thus meaningful for further analyses.

Iindigenous Galician populations showed poorer average performance than controls for most agronomic traits, such as rust susceptibility, aftermath heading and vigour. The best populations however performed as well as cultivars, particularly for early spring growth and autumn regrowth.

The correlation coefficients between agronomic or ecological traits and the first three principal components, which explained 70 % of the total variance are listed on table 2.

The first component gave high positive correlations (0.73 to 0.87) with seasonal growth parameters. High correlations (0.68, 0.56) were also shown by the first component and evapotranspiration (ETP) and average temperature of the coldest month (AmT). The second component is negatively correlated with time to flowering (HED) and rust susceptibility and is positively correlated with pH and calcium content, but negatively with aluminium content for the traits related to site habitat. Aftermath heading contributes most to the third component but there is no correlation with ecological traits.

The tree obtained by hierarchical clustering from the first four principal components scores is given in figure 1. Partition into 4 clusters was chosen by observation only. Clusters 1 and 4 and clusters 2 and 3 are most closely related to each other. The ratio of between cluster variance to total variance is 0.57.

The mean values of each cluster for the 12 agronomic traits are presented in table 3, in comparaison with overall mean and controls. Populations of cluster 4 show the most agronomically valuable scores for seasonal vigour and crown rust resistance and with the exception of aftermath heading are not dissimilar to the commercial variety controls. The data of table 3 were summarized and illustrated by projecting the cluster number of each population using the first two principal components as axes (Figure 2). The first component on the

Tabla 1.—RESUMEN ESTADISTICO Y CUADRADOS MEDIOS DE DOCE OBSERVACIONES AGRONOMICAS

Table 1.—Summary statistics and mean square analysis of the 12 agronomic traits

Traits	Galician Populations		Controls		Mean Squares	
	Average	Range	Average	Range	Populations	Error
Growth Habit	5,84	4,70 — 6,72	4,83	4,73 — 4,95	4,1 **	1,0
Tillering	6,10	4,73 — 7,30	6,69	6,31 — 7,40	7,5 **	2,2
Autumn Crown Rust	2,29	1,33 — 3,92	1,54	1,14 — 1,72	10,9 **	1,6
Autumn Growth	5,24	4,30 — 6,37	6,05	5,73 — 6,73	6,5 **	1,6
Early Spring Growth	7,32	6,48 — 8,32	6,90	6,55 — 7,19	12,5 **	2,5
Spring Growth	5,25	4,14 — 6,87	5,33	5,09 — 5,64	9,9 **	1,9
Spring Grownth Rust	5,12	4,02 — 6,12	4,33	3,93 — 5,28	7,9 **	2,6
Aftermath Heading	4,23	1,88 — 6,31	1,77	1,35 — 2,39	35,3 **	4,5
Summer Growth	5,40	3,79 — 7,47	6,22	5,31 — 6,61	12,6 **	3,0
Autumn Regrowth	4,58	3,27 — 6,93	4,48	3,92 — 5,57	11,49 **	2,4
Heading Date	145,84	126,77 — 158,29	129,60	118,10 — 139,80	1.343,80 **	43,8
Persistence	51,30	39,00 — 58,00	52,00	43,00 — 57,0	—	—

** Significant at the 1 % level.
** Significativo a nivel del 1 %.

Tabla 2.—COEFICIENTES DE CORRELACION ENTRE LOS COMPONENTES PRINCIPALES Y LAS VARIABLES INICIALES: OBSERVACIONES AGRONOMICAS (activas) Y VARIABLES ECOLOGICAS (ilustrativas)

Table 2.—Correlation coefficients between principal components and initial variates: agronomic traits (active) and ecological data (illustrative)

Agronomic traits	Principal Components			
	1	2	3	4
HAB	0,62	—0,52	0,03	—0,04
TIL	0,80	0,13	0,41	0,17
RUS	—0,33	—0,56	0,03	—0,66
AUG	0,87	0,11	0,36	0,10
ESG	0,73	0,39	0,05	—0,12
SPG	0,81	0,29	—0,07	—0,28
SRUS	0,03	—0,70	—0,16	0,54
AMH	—0,22	0,29	—0,80	0,00
SUG	0,76	0,05	—0,27	—0,05
AURG	0,77	—0,11	—0,49	—0,01
PER	0,50	—0,15	—0,53	0,04
HED	0,57	—0,67	0,08	—0,14
Ecological variates				
ASL	—0,54	0,11	0,01	—0,27
ETP	0,68	—0,13	0,04	0,13
R	0,10	0,08	0,47	0,03
AmT	0,56	—0,11	0,08	0,13
AMT	0,11	—0,07	—0,34	—0,14
Wdef	0,28	—0,07	—0,48	0,03
SAN	0,19	—0,22	—0,34	—0,07
LOA	—0,20	0,23	0,30	0,12
CLA	—0,08	0,10	0,35	—0,10
pH	—0,24	0,54	0,07	0,13
OM	0,18	0,10	0,28	—0,26
P	0,13	0,02	—0,24	—0,40
K	0,12	—0,06	—0,40	—0,09
Ca	—0,10	0,53	0,24	0,00
Mg	—0,03	0,28	0,15	—0,06
Al	0,03	—0,56	0,08	—0,04
% Cumulated variance	40,90	56,77	69,98	77,46
% Variance explained	40,90	15,87	13,21	7,48

x axis clearly separates clusters 2 and 3 from clusters 1 and 4; the latter includes the most vigorous, rust resistant populations. Late flowering populations are found on the negative side of axis y, i.e. in cluster 3 and partly in the large cluster 1. The four populations of cluster 4 represent the most valuable genetic material for short term breeding and perform as well as cv «Brigantia» (which itself originates from a native population from La Coruña).

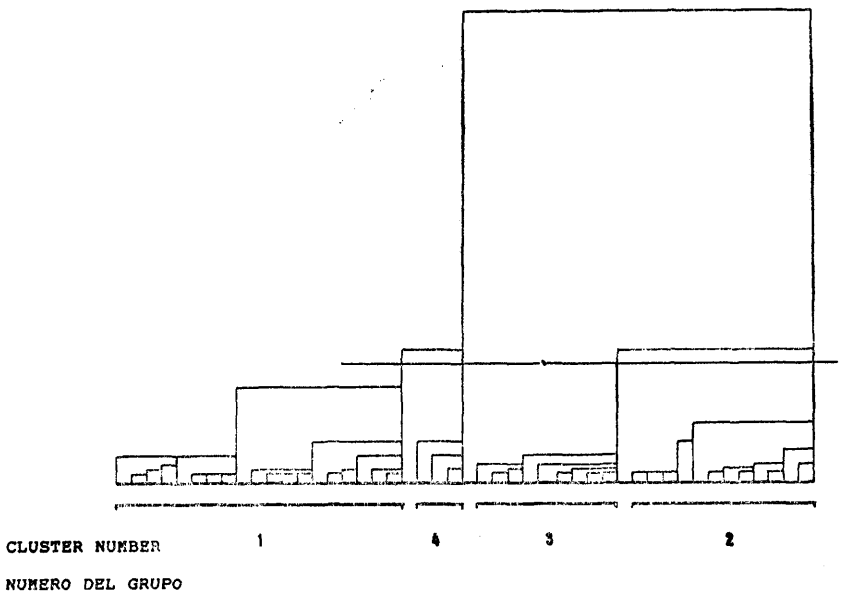


Fig. 1.—Dendrograma de las distancias (distancia euclidiana standard) entre cuarenta y siete poblaciones naturales de raigrás inglés a partir de doce observaciones agronómicas.

Fig. 1.—Dendogramme of the similarities (standardized euclidian distance) between 47 wild ryegrass populations from 12 agronomic traits.

The geographical location of each population is presented in figure 3, each site being illustrated by a symbol for each agronomic cluster.

Most populations of clusters 1 and 4 originate in the coastal regions whereas those of clusters 2 and 3 are mainly found in the inner mountains.

Table 4 summarizes the results of the factorial discriminant analysis of the agronomic clusters by the ecological traits. When tested by one way analysis of variance (between clusters/within cluster), the most significant variates are water deficit, pH, calcium content and

Tabla 3.—VALORES MEDIOS DE LAS DOCE OBSERVACIONES AGRONOMICAS DE LOS 4 GRUPOS, MEDIA GENERAL DE LOS CUATRO GRUPOS Y DE LOS TESTIGOS

Table 3.—Mean values of 4 clusters, overall average and controls for the 12 agronomic traits

Cluster Number	HAB	TIL	RUS	AUG	ESG	SPG	SRUS	AMH	SUG	HED	AURG	PER
1	5,52	6,53	2,33	5,59	7,44	5,51	5,16	3,48	5,69	150,18	4,76	52,68
2	6,22	5,88	2,02	6,07	7,26	5,89	4,90	4,37	4,96	134,41	4,08	48,07
3	6,02	5,43	2,77	4,58	6,87	4,80	5,42	4,92	5,04	146,95	4,37	52,00
4	5,61	6,53	1,86	5,87	8,06	6,47	5,04	5,61	6,44	148,55	6,02	54,75
Overall Mean	5,84	6,10	2,29	5,25	7,32	5,26	5,13	4,24	5,40	145,84	4,59	51,34
Controls	4,83	6,69	1,54	6,05	6,90	5,33	4,33	1,77	6,22	129,60	4,48	52,00

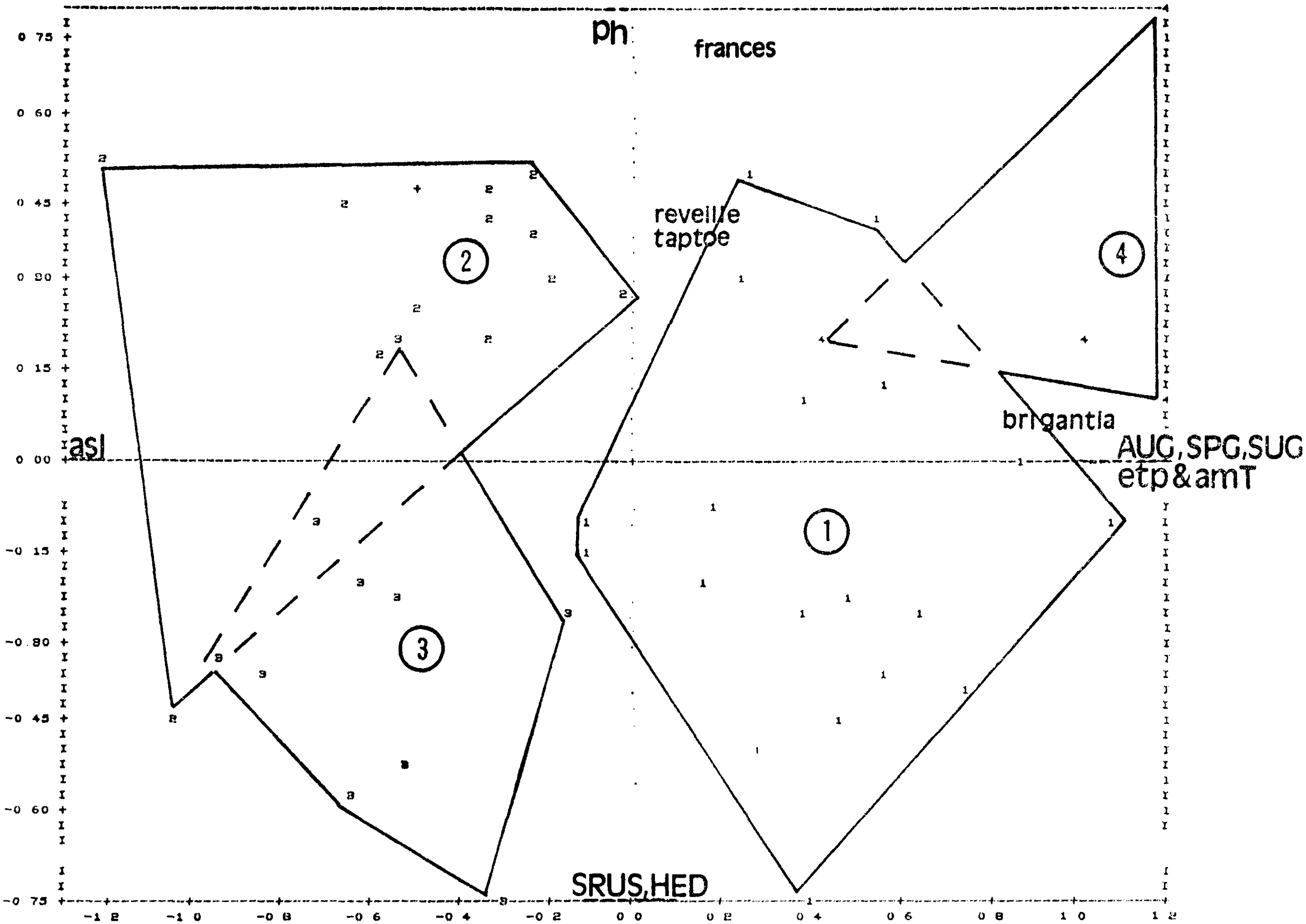
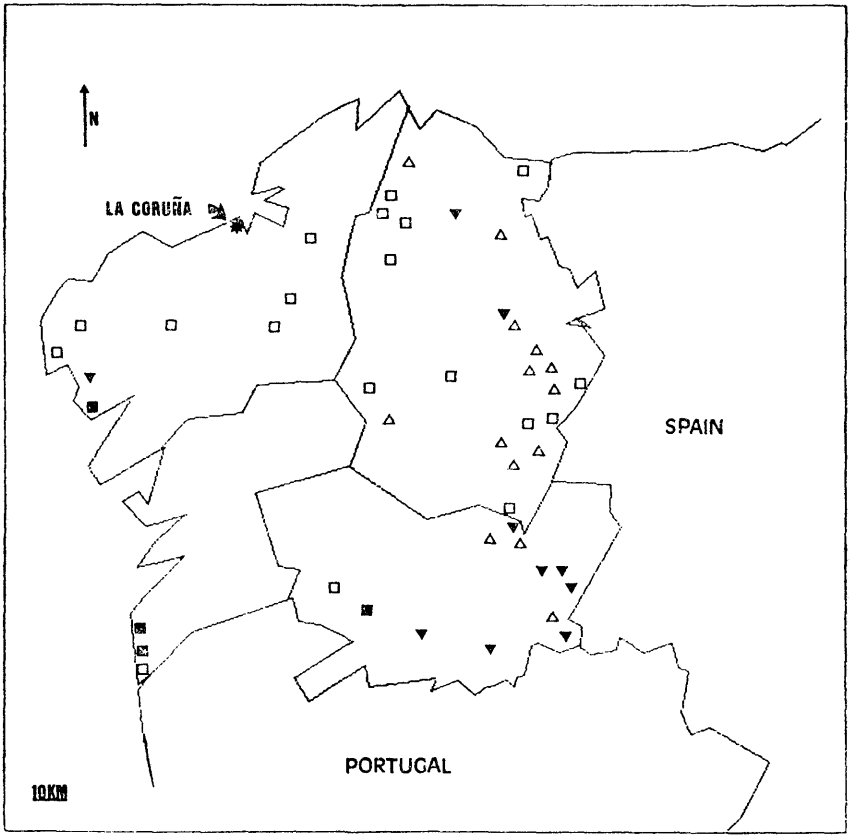


Fig. 2.—Proyección de las poblaciones de los cuatro grupos sobre el plano 1-2 del análisis en componentes principales principales (57 % de la varianza explicada).

Fig. 2.—*Projection of the populations of the 4 clusters on the plan 1-2 of the principal component analysis (57 % of the variance explained).*

potential evapotranspiration, six other ecological traits are significant at the 5 % level of probability (column univariate F). The discriminant factors are linear combinations of the initial variates and their F



CLUSTER	1 : □	GRUPO	1 : □
CLUSTER	2 : △	GRUPO	2 : △
CLUSTER	3 : ▼	GRUPO	3 : ▼
CLUSTER	4 : ■	GRUPO	4 : ■

Fig. 3.—Representación geográfica de las poblaciones de los cuatro grupos.

Fig. 3.—Geographical mapping of the populations of the 4 agronomic clusters.

value give an indication of their «discrimination power». The first two discriminant components are significant with F values much higher than any univariate F. Annual rainfall, organic matter in the

Tabla 4.—ANALISIS FACTORIAL DISCRIMINANTE: VALORES DE LA *F* DE FISHER DEL ANALISIS DE VARIANZA A UN FACTOR Y CORRELACIONES ENTRE LAS VARIABLES INICIALES Y LOS COMPONENTES *DISCRIMINANTES*

Table 4.—Factorial discriminant analysis: F values of one analysis of variance and correlations between initial variates and discriminant components

Traits	F (One way Analysis of variance)	Discriminant Factors 1	2	3
ASL	2,90 *	0,181	0,848	—0,501
ETP	3,22 **	—0,152	—0,939	0,314
R	3,86 *	—0,958	0,259	0,129
AmT	3,47 *	—0,457	—0,830	0,324
AMT	0,64	0,232	—0,803	0,552
Wdef	7,18 **	0,638	—0,768	0,085
SAN	2,76 *	0,509	—0,835	—0,215
LOA	2,50	—0,440	0,848	0,296
CLA	1,79	—0,714	0,695	—0,101
PH	5,58 **	0,079	0,702	0,708
OM	3,84 *	—0,998	—0,052	—0,049
P	1,29	0,061	—0,983	0,181
K	2,48	0,344	—0,868	—0,361
Ca	5,19 **	—0,342	0,698	0,630
Mg	0,79	—0,834	0,326	0,448
Al	2,72 *	—0,009	—0,423	—0,906

Discriminant Factor	% Variance explained	Pseudo F
1	39,8	31,43
2	35,8	23,19
3	24,4	10,40

*, ** Indicar la significación estadística a nivel del 5 % y del 1 % respectivamente.

*, ** *Indicate the statistical significance at the 5 % and 1 % level respectively.*

soil and magnesium content are highly correlated with the first discriminant factor. Potential evapotranspiration, average temperature, sand, phosphore and potassium content, altitude, loam content, pH and calcium content are highly correlated with the second discriminant factor.

The two factors explain 75 % of the total variance and a projection of the 4 clusters on the plan defined by these discriminant factors is illustrated on figure 4.

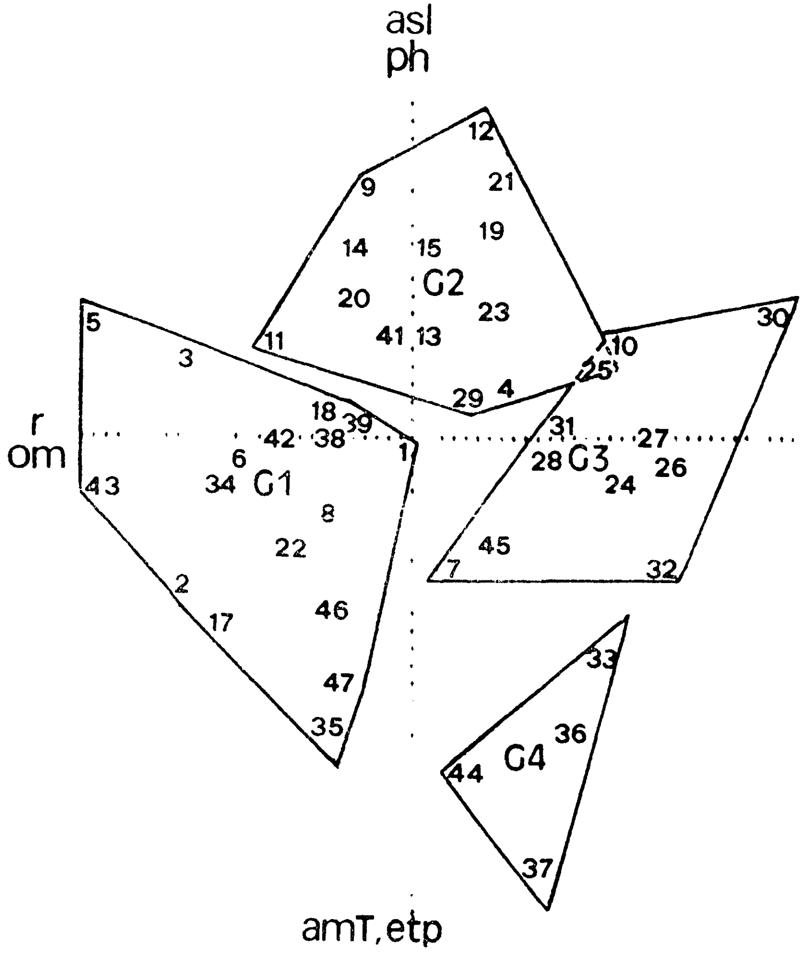


Fig. 4.—Proyección de las poblaciones sobre el plano 1-2 del análisis factorial discriminante: G1 a G4 indican los centros de gravedad de los 4 grupos agronómicos.

Fig. 4.—Projection of the populations points on the plan 1-2 of the Factorial discriminant analysis: G1 to G4 indicate the barycentres and the contours of the 4 agronomic clusters.

It is noteworthy to see that the four clusters made from agronomic traits are well discriminated by ecological features of their site of origin, with practically no overlapping. Moreover, the respective position of each cluster on this plan is quite different from that on the plan of the principal components (Fig. 2) and not deduced simply by a rotation. Indeed on the discriminant plan (Fig. 4), the first discriminant factor (x axis) opposes the populations of cluster 1 originating from sites, with higher rainfall, high content of organic matter and high magnesium in the soil to those of cluster 3 originating from dryer regions with high water deficit.

Cluster 2, the populations of which were derived from loamy soils at high altitude is contrasted on the second discriminant factor, to cluster 4, whose populations were found on sandy soil in warmer regions with hight potential evapotranspiration.

Discussion and conclusion

Wild populations of perennial ryegrass from Galicia show quite a wide range of variation for agronomic traits compared to the sample of cultivars investigated. Moreover the same appears to be situation for isozymes markers (thought to be neutral): preliminary results from 6 of the described populations (about 100 plants each) and 7 polymorphic loci (namely, phosphoglucose isomerase PGI, acid phosphatase ACP, esterase EST, phosphoglucomutase PGM, shikimate deshydrogenase SHDH, Glutamate oxaloacetate transaminase GOT 3, phosphogluconate deshydrogenase PGD) indicate average heterozygosity of 0.395, mean number of alleles per polymorphic locus of 3.16 and average of the Nei's distance for every pairs of populations of 0.057.

These parameters have intermediate values between those found by Hayward (1985) for indigenous ryegrass from Great Britain on the one hand and from Italy on the other hand.

This seems to support the existence of what might be called a «diversification center» of the genus *Lolium* in the mediterranean basin. The lower value of differentiation parameters in this material compared with that from Italy and the rareness of *L. multiflorum* traits foundian perennial ryegrass from Galicia (where both perennial and italian ryegrass coexist) suggests that Galicia would be on the northern margin of this presumed center of diversification.

Indeed the populations studied here are not too numerous to try to keep the wole collection in a cool chamber. The choice of a «core» of this collection would be dicted by the need of seed multiplication for distribution to other gene banks or further evaluation for yield in a multisite design. This sampling could judiciously by based on the mulivariate classification presented here as suggested by FALCINELLI et al., (1987), or PEETERS, MARTINELLI (1989).

Some populations should be picked up randomly in each clusters, with higlight to the most promising one, cluster 4. Such a way allows the conservation of every «phenotype» of ryegrass populations, but should be completed by genetic distance studies. It would be indeed interesting to know the cause of phenotypic similarity: genetic similarity (i.e. a common ancestor) or evolutionnary convergence du to the same selection pressure on different genetic backgrounds.

The relationships between agronomic and environment parallels the ecotypic adaptation found by other authors (BREESE, TYLER, 1986).

FORDE and SUCKLING (1980) found a linear relationship between vigour and P content of the soil, but this correlation was found only one year out of three of trials.

TYLER, CHORLTON (1982) found significant correlation between spring growth and heading date with the average March temperatures at origin. KOVACS (1984) succeeded in relating persistence and reproductive strategy (i.e. vegetative or sexual) of perennial ryegrass in Romania to coeno-ecological indices. More recently, FALCINELLI et al., (1987) linked altitude and annual rainfall to precocity in perennial ryegrass from Italy and TYLER (1987) established that late flowering perennial ryegrass was confined to a heavily trodden track while the surrounding population in a North Italian meadow was early flowering.

In our study, the correlation coefficients between individual agronomic and ecological traits are generally quite low, although some are significant when tested between clusters, so it is quite surprising that multivariate discriminant analysis using two linear combinations of ecological traits discriminate clusters established from agronomic traits only. These linear combinations, the discriminant components of factors could thus be called «ecological indices» and used for the choice of further sites for collecting population containing desirable characteristics. Further studies would be needed to establish what combination of selection features are responsible for the differentiation of agronomic traits, and whether it leads to a morphological convergence from a broad genetic basis or a narrow one.

Wild populations of perennial ryegrass from Galicia are likely to be interest to forage grass breeders, especially for use in mild winter regions like New Zealand or Britanny in France (EASTON et al., 1989).

This study will be continued by collection in other regions of North Spain Asturias, Cantabria, etc.) and indigenous material evaluated in a similar way.

In the short term, a recurrent selection programme has been initiated to make a broad base population by intercrossing plants from the best populations of cluster 1 and 4 to provide new cultivars well adapted for use in Galicia.

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RESUMEN

CARACTERIZACION DE POBLACIONES NATURALES DE RAIGRAS INGLES *(Lolium perenne L.)* DE GALICIA

En 1985 se han recogido en forma de semilla cuarenta y siete poblaciones naturales de raigrás inglés de Galicia y se evaluaron en el Centro de Investigaciones Agrarias de Mabegondo (22 km de La Coruña) en un campo de plantas aisladas. Se observaron doce características agronómicas entre 1986 y 1988.

Por término medio, las poblaciones naturales mostraron peores características agronómicas que los testigos. Sin embargo, debido a que mostraron mayor variabilidad, las mejores se comportan de forma similar a los testigos en algunas características agronómicas.

Se ha realizado un análisis en componentes principales sobre las medias poblacionales y se ha aplicado una clasificación ascendente jerárquica sobre las componentes principales. Esta clasificación condujo a la obtención de cuatro grupos de poblaciones, uno de los cuales reagrupó las poblaciones más interesantes. Es de destacar que el cultivar «Brigantia», obtenido por selección masal a partir de una población natural recogida en La Coruña aparezca como el mejor de los testigos en la evaluación.

Se realizó un análisis factorial discriminante sobre una serie de variables ecológicas (características del suelo y del clima) de los lugares de recolección de las poblaciones. Este análisis mostró que hay variables ecológicas que discriminan los grupos obtenidos a partir de las observaciones agronómicas.

Se discuten las implicaciones de estos resultados en la gestión de recursos genéticos y en la mejora genética de estas poblaciones.

Palabras clave: *Lolium perenne*, recursos genéticos, clasificación jerárquica, poblaciones naturales, variables ecológicas.