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**METHODOLOGY TO EVALUATE FORAGE LEGUMES FOR OVERSOWING
GRASSLANDS IN THE BASALTIC REGION OF URUGUAY**

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Abstract

A methodology used to evaluate around 300 temperate and subtropical forage legumes for oversowing the native grasslands of the Basaltic Region of Uruguay is presented in a four-year plan using the minimum amount of seeds per accession and resources. Row-column experimental designs are used to reduce the error variance existing due to the large soil heterogeneity intrinsic to the Region. The ability of the species to grow and reproduce was measured and adjusted least square means were estimated to rank them. Cluster analysis was also useful to group species with similar behaviour overall traits. Preliminary results for the temperate species showed that the methodology is useful for ranking and grouping a large number of forage legumes according to their overall trait performance

Keywords: Forage legumes, row-column design

Introduction

The practice of introducing forage legumes into existing native grasslands is considered a sustainable and environmentally friendly option to increase productivity in the production systems of the Basaltic Region of Uruguay. The Region is located in a transition zone in-between a temperate and subtropical climate with very distinct types of soils. Therefore, a large number of forage species might be potentially valuable for some of these heterogeneous climate and soil conditions. A methodology is proposed to evaluate forage legumes in competition with the native grasslands using the minimum amount of seed and resources.

Material and Methods

A four-year experimental procedure was planned to evaluate around 300 species (Table 1) in the Basaltic Region of Uruguay at Glencoe (Lat. 32° 01'32''S, Long. 57° 00' 39''W), Experimental Research Station of the National Institute of Agricultural Research (INIA) in 3 experimental sites with typical distinct soils: brown-reddish lithosols, black lithosols and paheozems/vertisols (vertisols) (FAO/UNESCO, 1970).

Five hundred accessions (temperate and subtropical species) per year were evaluated with different accessions of the same species every year. At the end of the experimental period, perennial and annual species would be of different ages or number of cycles of regeneration according to the year of sowing.

The large soil heterogeneity existing within each of these three testing sites makes difficult for the traditional experimental designs (such as incomplete blocks) to appropriately control the experimental error. Thus, beside the benefit of using an appropriate experimental design, it is expected that spatial analyses of the type proposed by Gilmour et al. (1997) would contribute to control the error variance. The experimental designs used in each of the sites were

row-column designs (Nguyen and Williams, 1993). For the temperate species were with 16 rows, 19 columns and 5 replicates and with 16 rows, 20 columns and 2 replicates. For the subtropical species were with 8 rows, 10 columns and 5 replicates and with 8 rows, 10 columns and 2 replicates.

To efficiently use the few seeds available per accession, 100 seeds are randomly selected and germinated following the International Seed Testing Association standards. Forty-eight seedlings are transplanted to trays, as a safeguard to obtain 21 plants required per accession. The two-size experiments (one with 5 replicates and one with 2 replicates), per species group and site, enables to use most of the accessions even the ones with less than 21 plants. For example using 1 plant per replicate, accessions with 6 to 14 plants could be included in the 2-replicate experiments, accessions with 15 to 20 in the 5-replicate experiments and accessions with more than 21 plants in both experiments. Subsequent analysis would allow combining information using all the accessions in common as well as the information of 4 control-accessions (*Trifolium repens*, *Trifolium pratense*, *Lotus corniculatus* and *Lotus subbiflorus* for the temperate group) and 2 control-accessions (*Lotononis bainesii* and *Desmodium incanum* for the subtropical group). Plants of each accession are sorted in the glasshouse and spare plants are left for seed multiplication. Individual plants of temperate and subtropical accessions are transplanted into the native grassland in a grid of 1m x 1m in July and in a grid of 1.5m x 1.5m in October, respectively.

Evaluation is done under a medium-level of fertilisation including N and P to allow them to express their potential overcoming the lack of appropriate rhizobium. A parallel rhizobium research program is accompanying these experiments to find appropriate bacteria able to nodulate, to fix nitrogen and to persist.

The outcome of these experiments is to identify forage species adapted to grow and reproduce in the required environment. Therefore, the main vegetative and reproductive traits measured per spaced plant are: (a) plant volume measured by maximum height (dm) and maximum spread (dm) in two directions for September and October and (b) total number of flowers produced in the growing season. Grazing and/or mowing is used at strategic times to manage defoliation.

The goal of the analysis is to use all the information to rank accessions (including the control accessions, for comparing and interpreting the results) based on the adjusted least square means (LSMEANS) obtained from a linear model analysis of variance, for each trait and all of them. Cluster analysis is used to form homogeneous within- and heterogeneous between-groups of accessions. Ward's minimum variance within group method for the Cluster Analysis was used. Adjusted means from the Spatial analysis (Gilmour et al, 1997) or the Row-Column design analysis were used in the cluster analysis. The Spatial analysis is recommended in the presence of large soil heterogeneity given the fact that the Row-Column design analysis can not always account for this and others variables experimental conditions. In the following example a mixed linear model (SAS, 1997) for the Row-Column analysis, assuming experiment and accession as fixed effects, and replication within experiment, row within replication-experiment, and column within replication-experiment, as random effects.

Results and Discussion

The use of the proposed methodology is illustrated with preliminary results obtained with temperate species in 1998. Differences between accession were significant in the mixed model analysis. The cluster analysis dendrogram was cut at the 3 cluster-level, as defined by the pseudo-F test, and the groups obtained were significantly different according to the canonical analysis

and the Mahalanobis distances among groups. Cluster size and means per trait and site are presented in Table 2. For Brown-reddish lithosols, 2 species had an outstanding production and total number of flowers that made them belong to a separate cluster. For Black lithosols, 38 accessions in clusters 2 and 3 had good productive and reproductive levels. For Vertisols, the 19 accessions of cluster 3 are the more productive and prolific ones.

Trifolium repens and *Trifolium pratense* were grouped in cluster 1 for the 3 sites showing a poor overall performance. *Lotus corniculatus* was grouped in cluster 1 for the two lithosol-sites and cluster 2 for the vertisol-site showing a relative improvement comparing with other accessions as the soil provides better growing conditions. *Lotus subbiflorus* was grouped in cluster 2 for the two lithosol-sites and cluster 3 for the vertisol-site showing a general better production and flowering potential than the other control species.

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Table 1 - Genus and number of species in evaluation for oversowing grasslands of the Basaltic Region in Uruguay

Genus	Number of Species	Genus	Number of Species	Genus	Number of Species
Adesmia	3	Galega	2	Neonotonia	1
Aeschynomene	2	Genista	5	Onobrychis	2
Albizia	1	Gleditschia	1	Ononis	2
Alysicarpus	1	Gliricida	3	Ornithopus	5
Anthyllis	4	Hedysarum	1	Retama	1
Astragalus	1	Hippocrepis	2	Rhynchosia	2
Biserrula	1	Lathyrus	9	Robinia	1
Cajanus	1	Lespedeza	4	Scorpiurus	1
Calicotome	1	Leucaena	2	Sesbania	1
Centrosema	1	Lotononis	1	Stylosanthes	4
Coronilla	1	Lotus	57	Sutherlandia	1
Cytisus	4	Lupinus	7	Tetranoglobulus	1
Chamaecytisus	1	Macroptilium	2	Trifolium	89
Desmanthus	2	Macrotyloma	2	Trigonella	8
Desmodium	7	Medicago	25	Vicia	13
Dorycnium	3	Melilotus	3	Vigna	3
Galactia	1	Mimosa	3	Zornia	1

Table 2 - Means per site and cluster for Volume in September and October and Total number of Flowers for temperate species in 1998

Sites	Cluster	Volume in September (dm ³)	Volume in October (dm ³)	Total Number of Flowers	Number of species per cluster
Brown-reddish lithosols	1	0.889	4.275	14.882	267
	2	1.367	7.989	126.761	43
	3	3.263	9.982	320.372	2
Black lithosols	1	1.102	5.478	22.341	278
	2	1.552	10.311	297.407	17
	3	1.677	14.994	165.535	21
Vertisols	1	0.998	5.073	16.427	245
	2	1.437	9.484	112.378	50
	3	1.293	12.579	285.504	19