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Selection of identification traits in the 'Tierra de Campos' alfalfa ecotype through discriminant analysis

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SUMMARY – This research was initiated with a survey of the most significant alfalfa types of Tierra de Campos areas, collecting 56 seed samples. These samples were later analysed in the laboratory (seed), in the greenhouse (seedlings) and in field trials (plant) under irrigated and rainfed conditions for three years. At the same time, several varieties of different dormancy rates were used as controls. Discriminant functions were calculated for the groups identified by the cluster analysis with the objective of determining the best traits for the identification of the ecotype. Persistence, spring regrowth and rate of growth were the best.

Key words: Dormancy rates, dry land conditions, *Medicago sativa* L., north-central Spain.

RESUME – *"Sélection des caractères d'identification de l'écotype de luzerne 'Tierra de Campos' par analyse discriminante". Ce travail a commencé avec une prospection dans les zones de Tierra de Campos où la luzerne est la plus cultivée, en ramassant 56 échantillons de semence, qui furent analysés en laboratoire (semence), serre (plantule) et champ (plant), en deux conditions différentes : en sec et en irrigation pendant trois ans. Des échantillons de différente dormance furent utilisés comme témoins. Avec toutes les données on a effectué une analyse Cluster, en distinguant clairement quatre groupes. Finalement, on a réalisé une analyse discriminante, en utilisant toutes les données de tous les caractères, pour savoir lesquels identifient mieux l'écotype. Ceux-ci ont été : persistance, repousse printanière et rythme de croissance.*

Mots-clés : *Dormance, conditions sèches,* Medicago sativa *L., nord-centre de l'Espagne.*

Introduction

The area of Tierra de Campos extends over the provinces of Palencia, Valladolid, Zamora and León in north-central Spain (Fig. 1). It is a large upland plain with continental climate and heavy soils. Cropping systems are mainly based on winter crops. Sheep foraging on the open fields and shrublands is by far the leading form of livestock raising. In spite of the large numbers of sheep in the area, forage production is not widespread and rainfed alfalfa, used for hay and pasture, is the main forage crop. The ecotype of alfalfa developed under these conditions is known as 'Tierra de Campos'. However the purity of the ecotype was considerably modified by the introduction of Flemish varieties.

Fig. 1. Tierra de Campos area in north-central Spain.

At present the seed market requires homogeneity and easy identification of the batches on offer. The objective of this research was to study the most suitable traits of the ecotype 'Tierra de Campos' for identification purposes.

Materials and methods

This research was initiated with a survey of alfalfa seeds collected in different areas of Tierra de Campos where rainfed alfalfa has traditionally been grown. Fifty-six samples of seed were collected and several important morphological, physiological and agronomic features (Delgado, 1989; Cordero and Crespo, 1995; Crochemore *et al*., 1998; Pecetti *et al*., 1999) were studied.

The study was conducted in laboratory, greenhouse and field trials, and several features were analyzed in every situation. The list of the characteristics studied and its frequency is presented on Table 1.

Table 1. List of analyzed characters and frequency of assessment per year

	1993		1994		1995		1996	
Plant	Dry	Irr.	Dry	Irr.	Dry	Irr.	Dry	Irr.
Spring regrowth (A11)		1	1	1	1	1	1	
Rate regrowth before 1st cut (A12, A13)		2	2	2	2	1	1	
Rate regrowth (A2,A3, A4, A5)	1 (A5)	5	4	4	4		2	1 (A5)
Flower color		1	1	1	1			
Flowering date		1	1	1	1			
Number of plants							1	1
Seedling		Spr.	Aut.					
Rate germination		1	1					
First leaf		1	1					
First trifoliate leaf		1	1					
Second trifoliate leaf		1	1					
Third trifoliate leaf		1	1					
First ramification		1	1					
Size of primary leaf		1	1					
Seed								
Size							1	
Weight							1	
Hardness							1	

The field trials with all samples collected and with several checks were carried out under both irrigation and rainfed conditions. The statistical design was a completely randomized block with three repetitions. The elemental plot consisted of 20 plants distributed in 10 plants per row, 0.70 m apart and 0.50 m between plants on the same line. Plots were separated by corridors of 0.80 m. This disposition allowed the least competition between plants.

Data were obtained from individual plants as well as from all plants in each plot, as Márquez *et al.* (1999) recommended when dealing with differences in the same ecotype.

Considering the high number of samples to be assessed and the large number of data collected from each sample, groupings and classes have been chosen and Cluster analysis was conducted, using Ward's method (Yamada and Suzuki, 1975; Crochemore *et al*., 1998; Pecetti *et al*., 1999). Once the groups were formed, analysis of variance for the traits was conducted, using the average values in each plot.

Finally, we performed a discriminant analysis with the objective of determining the traits best influencing on the classification as well as the distance between classes and the concordance between both groupings (achieved by cluster analysis and discriminant analysis). The analysis was performed using data from all traits. The implementation of discriminant analysis can be found in Rumbaugh *et al*. (1988), Cordero and Crespo (1995) and Dehghan-Shoar *et al.* (1997).

Results and discussion

Cluster analysis clearly differentiate four classes or groupings, Class 1 (C1) includes the earliest samples together with 5 samples collected in the region. Class 2 (C2) is the largest, with 35 samples of the region and the control 'Tierra de Campos'. Class 3 (C3) includes 9 samples. Finally, Class 4 (C4) is made up of the latest controls and 7 samples (Table 2).

Table 2. Arrangement of the samples and controls in every class

	Class 1	Class 2	Class 3	Class 4
Controls	Aragón, Sprinter, Moapa, Cuf 101, Baraka	Tierra de Campos		Cinna, Europe, Verdal, Romagnola, Milfeuil
Samples of 'Tierra de Campos'	5 (9%)	35 (62%)	9 (16%)	7 (12%)

C1 show the fastest spring regrowth and the highest regrowth rate after cutting. C2 showed lower spring regrowth and lower rate regrowth than C1, especially in the late cuts. It also showed the lowest mortality rate. C3 presented the lowest persistence and also lower precocity rate than C1 or C2. C4 exhibited the lowest precocity rate as well as the lowest regrowth rate after being cut, especially after the third cut.

After performing discriminant analysis only with rainfed data, four variables showed higher variability: height A11, height A13, height A3 and persistence. 76.47% of the samples could be classified correctly applying these four variables (Table 3).

Table 3. Classification matrix of discriminant analysis in rainfed conditions

Class	Samples correctly classified with the four variables used in the discriminant analysis (%)	Class				Total
		C1	C2	C3	C4	
C1	72.72	8	2†	1†	0	11
C2	77.77	1†	28	4†	3†	36
C3	55.55	0	3†	5	1†	9
C4	91.66	0	1†	0	11	12
Total	76.47	9	34	10	15	

†Number of samples whose initial classification does not coincide with the discriminant analysis and according to this the class to which it should belong.

The variables that exhibited the highest discrimination in field trials under irrigation conditions were height A13, height A2 and height A3. 72.05% of the samples could be classified correctly with these three variables. C4 achieves the highest percentage of correctness again. Using only these three variables in C1 we might make more errors when classifying samples (Table 4).

Table 4. Classification matrix of discriminant analysis in irrigated conditions

Class	Samples correctly classified with the four variables used in the discriminant analysis (%)	Class				Total
		C1	C2	C3	C4	
C1	45.45	5	3†	3†	0	11
C2	69.44	8†	25	2†	1†	36
C3	88.88	0	1†	8	0	9
C4	91.66	0	1†	0	11	12
Total	72.05	13	30	13	12	

†Number of samples whose initial classification does not coincide with the discriminant analysis and according to this the class to which it should belong.

When all data was used, 9 variables were found to discriminate between plants: dry land, height A11 and height A13, irrigation, height A1, height A2, height A3, height A4 and persistence, apart from measures of the primary leaf in the greenhouse trial. Wilks' Lambda factor was 0.04. Using all 9 variables, the coincidence between the initial consideration and the one obtained by discriminant analysis was of 91.04% (Table 5).

Table 5. Classification matrix using all the data after having performed discriminant analysis

Class	Samples correctly classified with the four variables used in the discriminant analysis (%)	Class				Total
		C1	C2	C3	C4	
C1	81.81	9	2†	0	0	11
C2	94.44	1†	34	1†	0	36
C3	77.77	0	1†	7	1†	9
C4	100.00	0	0	0	12	12
Total	91.04	10	37	8	12	

†Number of samples whose initial classification does not coincide with the discriminant analysis and according to this the class to which it should belong.

Conclusions

Four groups where established: early alfalfa (C1); late alfalfa, Flemish type (C4); 'Tierra de Campos' (C2); and a fourth type with intermediate traits between C2 and C3.

Not all samples were homogeneous. While some of them showed traits considered as representative of the ecotype 'Tierra de Campos' (62% of collected samples), traits being observed in other samples showed a mixture of alfalfa from other locations (17%) or other origins (21%).

The values of the traits that define the ecotype, such as spring regrowth, growth speed, flowering dates, growth rate and persistence, coincide with the samples that belong to the ecotype.

Height and persistence showed to be the best characters for discrimination purposes. While in rainfed conditions height has to be measured right from the start of the growth season, under irrigation conditions it has to be done in the middle of the vegetative growth.

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