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Characterization and selection of 'Tierra de Campos' lucerne ecotype using a method of identification of new varieties

M.R. Morales Corts*, S. Álvarez Sánchez-Arjona* and M.C. Crespo Martínez**
*FCAA, Univ. de Salamanca, Avda. Filiberto Villalobos 119, 37007 Salamanca, Spain
**SIDTA, Junta de Castilla-León, Apartado Oficial, 37080 Salamanca, Spain

SUMMARY – For three years a process of characterization and selection of 97 'Tierra de Campos' samples was carried out. Six samples were selected as the most productive and homogeneous regrowth and high leaf/stem ratio. Three of them were suitable for harvesting and the other three were suitable for grazing. A new selection was then made on the six selected lines, seeking the homozygotic ones for different alleles of the *locus Pgi-2*. These homozygotic groups can be used as mother plants for future synthetic varieties and would incorporate a kind of identification that would differentiate them from other commercial varieties, which would be polymorphic for this isozyme.

Key words: Characterization, homozygotic, isozyme.

RESUME – "Caractérisation et sélection de l'écotype 'Tierra de Campos' à l'aide d'une méthode d'identification de nouvelles variétés". Pendant trois années on a fait un travail de sélection et caractérisation de 97 échantillons de luzerne 'Tierra de Campos'. On a sélectionné six échantillons pour leurs hautes productions, repousse homogène et haute relation feuille/tige. Entre ceux-ci trois échantillons furent sélectionnés pour être fauchés, et les autres, pour consommation directe par les animaux. Après cela on a fait une nouvelle sélection en choisissant des plantes homozygotes pour différents allèles du locus Pgi-2. Ces groupes homozygotes peuvent être utilisés comme matériel de base pour l'obtention d'une nouvelle variété synthétique qui incorporerait une identification pour la différencier des autres variétés.

Mots-clés : Caractérisation, homozygote, isozyme.

Introduction

The alfalfa ecotype 'Tierra de Campos' is characterized by its cold and drought resistance and by its persistence (Hidalgo and Martínez, 1985), so it is rainfed-grown, with mixed use: for harvesting, grazing and seed production. The official variety characterization of alfalfa, based on morphological and physiological characteristics offers no clear differences between varieties, which means that doubts often occur in their identification (Ben Chaabane, 1990). Characterization studies of alfalfa varieties have been made with RAPDs (Brummer *et al.*, 1995), RFLPs (Kidwell *et al.*, 1994) and with isozymes (Brunel, 1982; Morales *et al.*, 1997). These studies cannot differentiate varieties that are genetically close, so it will always be difficult to distinguish a new synthetic variety from a parent line.

Facing with this situation, the present study suggests that when a program is developed for selecting and obtaining a synthetic variety in allogamous species the use of isozymatically uniform plants as mother plants may be of great interest (Douglas, 1983). However, it is necessary to confirm that a variety selection that incorporates a homogeneous expression for an isozyme does not change crop characteristics.

The objective of this study was to show some important characteristics of the 'Tierra de Campos' ecotype and to confirm that a variety selection, which incorporates a homogeneous expression for an isozymatic locus, does not change alfalfa characteristics.

Materials and methods

97 samples of seeds from dry farmed 'Tierra de Campos' alfalfa were selected. They were sown in three different locations during three years and the following characteristics were studied: weight of 1000

seeds, seed color, days to flowering, flower color, plant aspect, length of main stem, number of nodes on the main stem, length and width of leaves, new growth vigor, regrowth rate, leaf/stem ratio, crude protein contents and dry matter production.

After the characterization of the samples, 6 lines over 97 were chosen, as the most productive, with the most homogeneous resprouting, the highest leaf/stem ratio and the least damaged by frost. Two different groups were differentiated according to the aspect: the most erect lines as more appropriate for harvesting and those with the highest percentage of semi-erect plants, as appropriate for grazing. Using this material, in 1991, 6 rows of 120 plants per row were sown. Moreover, two plots of 3000 m² each were dry sown. One of them was sown with a mixture of the three lines selected for harvesting, and was subjected to this use for 4 years, and the other plot was sown with a mixture of the three lines selected for grazing, and subjected to continuous grazing. In spring 1995, 100 plants were selected from each of these plots. In the plot destined for harvest, plant erectness, high leaf/stem ratio and plant health were taken into account. In the grazed plot we selected those which were short, had a high leaf/stem ratio and healthy. All selected plants and the 120 plants of the six lines of 'Tierra de Campos' sown individually in rows and analyzed using starch gel electrophoresis to determine their genotype with respect to *locus Pgi-2* in 1995.

At the beginning of 1996, 7 microplots with 15 homozygotic plants on each group were established. The groups were the following: dddd harvest, dddd grazing, bbbb harvest, bbbb grazing, aaaa harvest, aaaa grazing and a'a'a'a' harvest and the characteristics used for ecotype characterization were measured.

Using the Statgraphic-Plus program a study of the quantitative traits for each group was made, calculating means and standard deviations. Analysis of variance was used to determine whether there were differences between groups for these types of traits. As to the qualitative traits, the frequencies were calculated and these were contrasted between the different groups using χ^2 analysis.

Results and discussion

From the 97 samples of 'Tierra de Campos' analyzed, 22 were considered out of type. Means and standard deviation of the studied parameters are presented in Table 1, showing great variability among samples and within each sample.

From the 920 plants analyzed for *Pgi-2* genotype we obtained 9 genotype aaaa plants, 13 bbbb genotype plants and 3 dddd genotype plants. By crossing selected plants for grazing and plants for harvesting that presented heterozygotic genotypes with 2 or 3 dosage of the same allele, we obtained new homozygotic plants for these three alleles and also the a'a'a'a' genotype that we did not find in cultivated fields. We did not get the cccc ecotype neither a'a'a'a' plants selected for grazing. The mean values and standard deviations of the parameters analyzed in the groups of 15 homozygotic plants for different alleles of *locus Pgi-2*, from the selections for harvesting and grazing, are given in Table 1.

The mean values of the analyzed characteristics are within the ranges of the global ecotype characterization. From ten quantitative characteristics analyzed, only 1000 seed weight and main stem length presented differences between the means of the seven groups. In this case differences were not due to the *Pgi-2* genotype. For main stem length, selection by use being more determinant, since the plants for grazing showed smaller values than those for harvesting. The global average of the main stem length for 'Tierra de Campos' was of 62.7 cm, although this value was improved by alfalfa selected for harvest.

In general, 'Tierra de Campos' behave as semi-dormant alfalfa, and the homozygotic groups followed this tendency whereas the grazing groups had somewhat slower regrowth than the harvested groups. 'Tierra de Campos' shows a shoot growth somewhat slower than Aragón and Flemish type alfalfa, although some samples of the harvesting groups have taller regrowth, almost 15 cm in height, 12 days after the first cutting.

Leaf to stem ratio has generally been considered a feature to improve in alfalfa, with 'Tierra de Campos' showing higher values than Aragón or Flemish types four of the selected groups show a higher mean than that of the ecotype (0.64). The leaf/stem ratio is related to the protein content, due to the fact that the leaf shows higher values than the stem. Our plants showed protein values of 19% to 23.7%, in

alfalfa plants just before the beginning of flowering. The predominant seed color in most of the groups is chestnut and amber. We consider that the differences found in seed color are mainly due to the stage of maturation at the time of collection and not due to genetic causes. Despite the fact that white and variegated flowers may appear in Tierra de Campos, we only found in the homozygotic groups light blue and dark blue flowers. As might be expected, the groups selected for harvesting showed plants with erect and semi-erect phenotype, whereas those selected for grazing showed semi-erect and creeping phenotype.

Table 1. Values of some parameters for 75 samples of alfalfa 'Tierra de Campos' and for selected homozygotic groups

Parameter	Global 'T. Campos'		a'a'a'a' harvest		aaaa harvest		bbbb harvest		dddd harvest		aaaa grazing		bbbb grazing		dddd grazing	
	Mean		Mean		Mean		Mean		Mean		Mean		Mean		Mean	
1000 seeds (g)	2.38	0.12	2.49	0.37	2.41	0.08	2.33	0.28	2.51	0.33	2.17	0.14	2.18	0.23	2.13	0.26
Seed colour																
% chestnut	28		0		50		25		16		12		58		50	
% green	5		66		0		9		9		0		25		10	
% brown	11		0		0		0		0		0		8		20	
% amber	56		34		50		66		75		88		9		20	
Days to flowering from 1-05	33.1	3.2	35.25	2.19	35.5	0.71	34.33	2.53	36.41	2.71	37.36	1.38	33.41	6.91	36.72	1.79
Flower color																
% white	0.8		0		0		0		0		0		0		0	
% variegated	3.5		0		0		0		0		0		0		0	
% light blue	62.8		0		50		17		83		27		83.3		9	
% dark blue	32.2		100		50		83		17		73		16.6		91	
Plant aspect																
% erect			50		0		83.3		83.3		18.8		0		0	
% semi-erect	19.5		50		100		16.7		16.7		63.64		25		60	
% creeping			0		0		0		0		18.18		75		40	
Length of main stem (cm)	62.7	8.8	68.12	2.53	74	4.24	76.5	13.67	68.66	11.8	66.27	9.39	66.58	8.08	53.36	12.78
No. of nodes on the main stem	14.5	3.3	13.62	1.18	15.5	4.94	15.17	2.29	15.41	1.97	15.45	2.07	14.50	2.11	14.27	2.00
Length of pinnule (mm)	25.2	2.3	24.25	6.32	27.5	3.53	26.33	5.38	28.00	5.48	25.27	3.29	23.16	5.32	22.81	3.57
Width of pinnule (mm)	12.2	2.8	12.00	1.93	12.50	0.71	12.33	3.36	12.00	2.17	11.73	0.90	13.16	5.13	12.54	2.38
Regrowth rate (after first cutting)	13.9	4.9	15	5.34	14.5	0.71	16.36	2.94	12.5	6.46	13.72	5.14	13.42	2.10	10.50	4.03
Regrowth vigor	5.8	-	6.62	0.52	6.75	0.35	5.36	1.28	6	1.54	6.09	1.64	6.33	1.15	5.5	1.84
Leaf/stem ratio (g d.m./g d.m.)	0.64	0.12	0.55	0.21	0.71	0.28	0.79	0.2	0.60	0.11	0.80	0.09	0.77	0.23	0.60	0.11
Protein contain: % d.m. aerial parts	20.03	2.22	21.25	2.33	21.70	1.06	23.75	2.94	20.22	1.3	21.56	1.05	20.5	0.63	19.02	0.92

Conclusions

The results confirm that the grouping based on the type of use, harvest vs. grazed, does not make physiological and crop quality characteristics vary. Thus, we consider that when a program for selecting and obtaining a synthetic variety in allogamous and polyploid species is developed starting from a limited number of parent plants, it may be of great interest to seek groups that are isozymatically uniform for a polymorphic *locus*, which makes it possible to differentiate perfectly the new variety obtained, even from those genetically closest

We obtained four groups of plants homozygotic four different alleles of *locus Pgi-2* selected for harvesting and three groups selected for grazing. The best of each of them could be used as parent plants

for a synthetic variety of the Tierra de Campos ecotype, which could be perfectly differentiated from the rest of varieties.

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