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Performance of *Lotus corniculatus* L. genotypes submitted to cutting interval: subsidies to a breeding program

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ABSTRACT - The objective of this work was to evaluate the response of five birdsfoot trefoil populations (*Lotus corniculatus* L.), selected under severe and intense grazing (P38, P37, P9 and P3) or cutting (population Corte), one rhizomatous population from Morocco and two cultivars, São Gabriel (Brazil) and ARS 2620 (USA), to different cutting intervals (20 and 40 days). The trial was carried out in a greenhouse for 224 days. The plants were submitted to the treatments during four months, when it was made four and two cuttings for the 20 and 40 day intervals, respectively. In the following months, aerial part of the plants was totally removed and after 70 days of regrowth, evaluation of roots and aerial section were performed. Analysis of variance and multivariate analysis was performed, obtaining the Mahalanobis distance (Md), dendogram by UPGMA method and the relative contribution of the characters for genetic divergence. There was no genotype × cutting interval interaction and the genotypes presented a reduction on dry matter yield of roots, crown and aerial sections, number of stems and plant height when submitted to frequent cuttings. The most divergent genotypes were Marrocos and P9 (Md = 108.7) and the most similar ones were São Gabriel and P37 (Md = 7.8). The results suggest exclusion of the population P9 because of its weak performance and the utilization of the populations Corte and P37 as progenitors in the birdsfoot trefoil breeding program. Root dry matter accumulation and plant height were the characters that contributed most to genetic divergence and they can be used for selection works.

Key Words: birdsfoot trefoil, forage production, management, root

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is a crown-former legume and it depends on individual survivals to persist in pastures. In grazing situation, the stand persistence depends on the management to ensure the natural reseeding and the establishment of news seedlings.

In Brazil, the species is cultivated in the Southern states, where the subtropical climate is more appropriate for its development and the production cycle is concentrated in the spring-summer season. Since its introduction in Rio Grande do Sul in the 1960s, the unique commercial cultivar available is São Gabriel, whose erect growth habit makes its survival under grazing difficult. However, to Soster et al. (2004), cv. São Gabriel varies for growth habit and the selection for better persistance may be possible. In Uruguay, the main restriction for forage productivity is the lack of persistence (Rebuffo, 2005) and Altier (1997) pointed at the root rot complex (*Fusarium*, *Colletotrichum*, etc) as the main reason for this problem with cv. São Gabriel.

The evaluation of morphological variability and the regrowth potential of birdsfoot trefoil genotypes as function of cutting interval can offer information for selection in breeding programs. Flaresso & Saibro (1992) and Araújo & Jacques (1974) verified a better response of this cultivar to low defoliation frequency. In this situation, the birdsfoot trefoil showed greater dry matter yield, higher non-structural carbohydrates content, better natural reseeding and survival in relation to high defoliation frequency. Santiñaque & Batista (2003) also observed an increase of 45% in forage production using a 44-day cutting interval compared to the 22-day cutting interval.

Morphological characterization is an important procedure to identify desirable traits of progenitors to be included in breeding programs (Oliveira & Valls, 2003) as well as the estimation of genetic diversity. A knowledge of genetic distance not only produces a better understanding of germplasm organization and higher efficiency during genotype sampling but also it results in the biologically oriented choice of crosses and gene introgression from exotic germplasm, and it can also be used to recommend

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cultivars when the goal is to increase the genetic basis of commercial cultivars for a given region (Vieira et al., 2007).

The objective of this study was to compare the performance of birdsfoot trefoil genotypes under cutting intervals management in order to select the better genotypes to be used in our breeding program.

Material and Methods

The experiment was carried out in a greenhouse, during seven months, in Passo Fundo, Rio Grande do Sul, at 687 m of altitude, with subtropical climate (Figure 1).

In this trial, the effect of cutting interval (20 and 40 days) in eight birdsfoot trefoil genotypes, being five populations derived from cv. São Gabriel (Brazil), a population from Morocco, named Marrocos, and cvs. São Gabriel and ARS 2620 (EUA) were evaluated. The populations derived from cv. São Gabriel were selected by submitting them to a heavy grazing pressure (P38, P37, P9, P3) or to a severe and frequent cutting (population Corte). The treatments were arranged in a complete randomized design with five replications.

The plants were obtained by seed germinating and transplant the seedlings to pots containing the substrate with the following attributes: organic matter = 1.1%; pH = 5.2; P = 16 mg/L; K = 132 mg/L; S = 58%; CTC = 12.9 $cmol_c$/L. There was no need for fertilization because the chemical conditions of the substrate were in accordance to the specie requirements (Comissão…., 2004). Rizobia inoculation was done by using aqueous solution with specific strain after the transplant, in December 4^{th}, 2002. When the plants were 54 days of growth, the first cutting was realized, leaving a 7 cm long stubble. After this date, for the 20 days interval, the cuttings were made on January 28^{th}, 2003; February 17^{th}, 2003 (second cutting, first regrowth); March 7^{th}, 2003 (third cutting, second regrowth); March 28^{th}, 2003 (fourth cutting, third regrowth) and April 17^{th}, 2003 (fifth cutting, fourth regrowth).

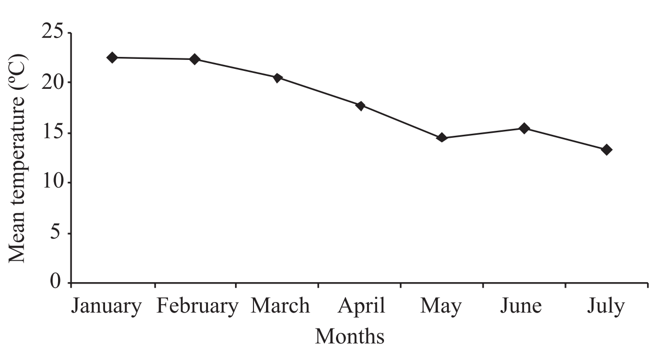

Figure 1 - Average outside temperatures of greenhouse during the experimental period.

For the 40 days interval, the cutting was made on January 28^{th}, 2003; March 7^{th} 2003 (second cutting, first regrowth) and April 17^{th},2003 (third cutting, second regrowth). After ending of the evaluating period, a new regrowth was allowed and a severe cutting at the ground level was made on May 5^{th}, 2003. After 70 days, on July 15^{th}, 2003, a final evaluation was performed. The plants were evaluated in relation to height, length of internodes on the tallest stem and shoot dry matter weight. In the first cutting and at the final evaluation, the number of stems was counted. After the first cutting, the plants were visually evaluated in relation to residual leaf area by grading them (1 = low; 2 = medium; 3 = high). In the final assessment, the material was taken out of the pots, the roots were washed in tap water and the crown diameter was estimated at the cotyledonal knot region. Later on, the plants were segmented into crown, shoots and roots, and the components harvested on each cutting were oven-dried (60ºC) until constant weight was reached.

Data were submitted to variance analysis, with means comparison by using the Tukey test (5%), and multivariate analysis, by determining the Mahalanobis distance, the relative contribution to the genetic divergence and grouping by the UPGMA method. The statistical software Sisvar (Ferreira, 2000) and Genes (Cruz, 2001) were used. For the multivariate analysis, 14 characteristics related to the regrowth shoot dry matter, plants height, and in the final evaluation the shoots, crown and roots dry matter yield, number of stems and the internodes length of the tallest stem were selected.

Results and Discussion

Considering the initial growth the period from planting to the first cut, the genotypes varied for stems, dry matter and residual leaf area, showing variability for all morphophysiological characteristics (Table 1).

In spite of the largest number of stems, cv. ARS 2620 showed a more prostrate and compact habit, characterized by short internodes. However, its great number of stems and residual leaf area did not result in the largest dry matter yield. Soster et al. (2004) obtained similar values for internodes length for the same genotypes of this experiment, and cv. ARS 2620 also showed the shortest internodes (1.71 cm).

On the other hand, P9 genotype, which presented the smallest number of stems, also had the smallest leaf area. The largest residual leaf area was for cv. ARS 2620, Corte and P37, which suggests a better regrowth potential.

Maroso et al. (2007) also observed larger residual leaf area in cv. ARS 2620 in relation to cv. São Gabriel. In general, cv. São Gabriel presented similar values to those found in the genotypes derived from it in the first evaluation, differing only from the P9 genotype for the number of stems and dry matter yield.

Although the genotypes had shown differences in the initial growth period, there was no genotype × cutting interval interaction. All the genotypes had a reduction in their morphological attributes when submitted to a shorter cutting interval (Table 2).

According to Smith & Nelson (1967), stubble height has more effect on the dry matter production of birdsfoot trefoil when the cuttings are frequent. Maroso et al. (2007) obtained greater dry matter yield of the cvs. São Gabriel and ARS 2620 under 30-day cutting interval in relation to 7-day cutting interval. Flaresso & Saibro (1992) verified that cv. São Gabriel was benefited by cutting interval of 6 weeks and 9 weeks in relation to 3-week interval, with greater dry matter yield, higher non-structural carbohydrates level and plant survival rate.

Populations of Corte and P9 were the most contrasting ones, with expressive superiority for the former. However, Soster et al. (2004) did not observe any difference among Corte and P9 genotypes submitted to cuts at every 45 days after flowering in a field evaluation. These conflicting results probably are due to the conditions of growth and the period of evaluation of the different trials. In the present study, the plants were grown in pots and cuttings were made early, at 54 days of age, whereas in studies of Soster et al. (2004) this was done in the maturity phase. Plants with different development stages show distinct responses to defoliation because of the number and accessibility of growing points, as well as the amount of organic reserves in the crown and roots. The weak performance of P9 can be partly attributed to its smallest residual leaf area after cutting and to the smallest number of stems showed during the initial growing phase (Table 1).

In the last harvest, genotypes of ARS 2620 and Corte showed the greatest number of stems, but they differed for height, expressing different growth habit, which were prostrate and erect, respectively (Table 3). Also in this evaluation, the population P9 showed the smallest values for most of the traits evaluated, being the only one to present a reduction on persistence (90%). The other genotypes did not show plant mortality during the trial.

The multivariate analysis pointed out that the longest Mahalanobis distance was between a population derived from the cv. São Gabriel (P9) and an exotic population from

Table 1 - Morphophysiological traits of birdsfoot trefoil genotypes at the first cut in plants at 54 days of age

Genotype	Stem (number/plant)	Dry matter (g/plant)	Internode (cm)	Residual leaf area*
Corte	7.6ab	5.6a	2.2ab	2.3ab
P37	8.3ab	3.7ab	2.1ab	2.1ab
São Gabriel	6.3b	3.9ab	2.4ab	1.7abc
ARS 2620	12.3a	2.8ab	2.1b	2.5a
P3	6.9b	3.3ab	2.3ab	1.9abc
P38	7.2b	3.8ab	2.6a	1.6bc
Marrocos	9.0ab	2.5b	2.2ab	1.9abc
P9	5.2b	2.3b	2.3ab	1.1c
CV (%)	31.1	21.9	15.8	31.0

Means followed by the same letter (within a column) do not differ by Tukey test at 5% of significance. *1 = low; 2 = medium; 3 = high.

Table 2 - Dry matter yield of birdsfoot trefoil genotypes in the means of cutting interval and genotypes

Genotype	Regrowths during application of cutting intervals (g/plant)	Total regrowth (g/plant)
Corte	8.4a	16.9a
P37	7.4ab	15.5ab
São Gabriel	7.2ab	13.1abc
ARS 2620	7.1ab	15.4ab
P3	6.2abc	13.1abc
P38	5.8abc	12.4abc
Marrocos	5.2bc	10.7bc
P9	4.5c	8.9c
Cutting interval		
20 days	4.2b	10.2b
40 days	8.4a	16.8a
CV (%)	15.6	13.7

Means followed by the same letter (within a column) do not differ by Tukey test at 5% of significance; CV = coefficient of variation.

Marrocos (108.7), whereas the most similar genotypes were São Gabriel and P37 (7.8) and ARS 2620 and P3 (7.9) (Table 4). Genetic distance can be an adequate parameter for choosing parental combinations in a crossing program (Cowen & Frey, 1987).

The relationships among the genotypes are illustrated in the dendrogram (Figure 2), with the formation of three groups: group 1, formed by the genotypes P31, São Gabriel and Corte; group 2 with the population P9 and the group 3, formed by the ARS 2620, P3 and P38 genotypes.

The groups formed reflected the agronomic similarity of genotypes in response to the cuttings. Marrocos and ARS 2620 rhizomatous genotypes registered the smallest DM yield in this study, agreeing with the results shown by Kallenbach et al. (2001), who also related the small yileds of rhizomatous types of birdsfoot trefoil, and 50% inferior to the genotypes without rhizomes. Group 1 showed the best agronomic attributes due to its greatest regrowth dry matter yield and to the largest number of stems, showed by the genotypes under the shortest cutting interval. The

Table 3 - Dry matter yield and morphophysiological traits of birdsfoot trefoil genotypes at the final harvest

Genotype	Root (g/plant)	Crown (g/plant)	Shoot (g/plant)	Total (g/plant)
Corte	4.3a	1.3ab	3.3a	8.9a
P37	3.5ab	1.1bc	3.0ab	7.5ab
São Gabriel	3.3abc	1.2 abc	2.9ab	7.5ab
ARS 2620	3.4ab	1.7a	2.5abc	8.2ab
P3	3.9ab	1.2abc	2.5abc	7.6ab
P38	3.1abc	1.2abc	2.1abc	6.4abc
Marrocos	2.4bc	1.1abc	1.6bc	5.4bc
P9	1.8c	0.6c	1.4c	3.8c
CV (%)	35.3	38.6	43.5	32.2

	Morphophysiological traits		
Genotype	Crown diameter (mm)	Stems (number/plant)	Height (cm)
Corte	5.1ab	118ab	15.4a
P37	4.6ab	110b	16.8a
São Gabriel	5.5a	81b	15.8a
ARS 2620	5.5a	178a	7.9b
P3	5.1ab	93b	17.0a
P38	4.9ab	96b	15.1a
Marrocos	4.7ab	108b	7.6b
P9	3.7c	64b	15.6a
CV (%)	22.6	43.8	22.2

Means followed by the same letter within a column do not differ by Tukey test at 5% of significance.

Table 4 - Mahalanobis distance matrix of birdsfoot trefoil genotypes submitted to cutting

Genotype	ARS 2620	Corte	P3	P37	P38	P9	Marrocos
Corte	49.1	-					
P3	7.9	67.5	-				
P37	46.6	21.8	51.4	-			
P38	20.6	87.2	13.8	75.6	-		
P9	75.2	52.0	71.7	47.4	61.1	-	
Marrocos	12.9	79.2	16.5	59.0	30.5	108.7	-
S. Gabriel	49.7	27.9	57.4	7.8	80.7	65.8	55.7

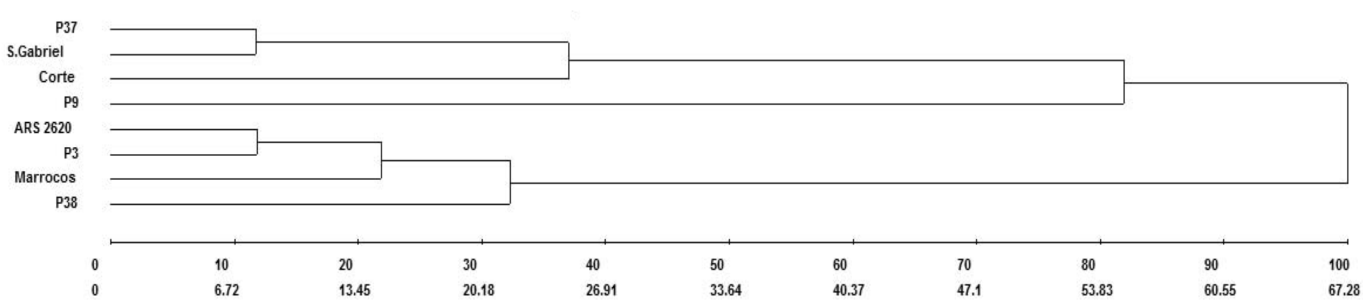

Figure 2 - UPGMA dendrogram based on Mahalanobis distance among eight genotypes of birdsfoot trefoil for morphophysiological characters.

genotypes in this group were always present among the most productive ones (Tables 1, 2 and 3), as well as they were among those with desirable forage characteristics (Table 5). Therefore, in order to give continuity to the breeding program, the populations P37 and Corte, due to their characteristics, should be the base of the new populations formed**.**

The character with the greatest relative contribution for the genetic divergence was the roots dry matter accumulation under the 20-day interval, followed by the plants height and the roots dry matter accumulation under 40-day interval, with a total of 62.75 of the germplasm divergence (Table 6). According to Assuero et al. (1992), root biomass yield is also consistent with the agronomic selection for persistence, because the roots are important as storage organs for survival and regrowth. Messa et al. (2007) identified wide variation in root characteristics for birdsfoot plants of cv. São Gabriel (Brazil) and Draco (Uruguay).

These results are very important if the type of selection made is taken into account and which results should be expected. Because the selection was made by imposing a severe stress (cutting or grazing) upon the material with high sensibility to this type of management, it should be expected that these characteristics were the most affected ones and consequently also it would allow a better discrimination of the germplasm. In other words, the selection made possible those differences.

Table 5 - Morphophysiological character of birdsfoot genotypes groups

Character	G1 (Corte, P37, S. Gabriel)	G2 (P9)	G3 (ARS 2620, Marrocos, P38, P3)
Regrowth dry matter production (20 days)	9.95	7.79	4.63
Regrowth dry matter production (40 days)	7.49	6.88	4.14
Total dry matter production (20 days)	3.68	3.12	1.98
Total dry matter production (40 days)	2.39	1.83	1.88
Dry matter root (20 days)	5.29	5.62	2.18
Dry matter root (40 days)	3.78	2.79	2.11
Crown dry matter (20 days)	1.70	1.60	0.90
Crown dry matter (40 days)	1.17	1.26	0.84
Stems at final harvest (20 days)	166.75	122.25	96.31
Stems at final harvest (40 days)	92.92	142.50	85.00
Mean plant height (20 days)	14.00	18.25	14.19
Mean plant height (40 days)	17.00	7.25	13.00
Internode at final harvest (20 days)	1.82	1.96	1.64
Internode at final harvest (40 days)	2.15	1.64	1.68

Table 6 - Relative contribution for genetic divergence (RCGD) by Singh's method (1985) in birdsfoot trefoil genotypes

Character	RCGD (%)
Regrowth dry matter production-20 days (R20d)	7.11
Regrowth dry matter production-40 days (R40d)	2.73
Total dry matter production (TDM-20 days)	2.46
Total dry matter production (TDM-40 days)	2.18
Dry matter root-20days	31.68
Dry matter root-40days	11.51
Crown dry matter-20days	6.34
Crown dry matter-40days	0.28
Stems at final harvest-20days	0.86
Stems at final harvest-40days	2.48
Mean plant height-20days	4.36
Mean plant height-40days	19.56
Internode at final harvest-20days	3.45
Internode at final harvest-40days	4.88

Conclusions

Cultivars and populations of birdsfoot trefoil from different origins do not differ in response to cutting interval, showing a drastic reduction on dry matter yield. Corte and P37 genotypes are valuable genotypes to be used as progenitors in the breeding program of birdsfoot trefoil. Root dry matter accumulation and plants height have important contribution to the germplasm divergence and genotype selection.

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