CARBON AND NITROGEN ISOTOPIC DISCRIMINATION IN ALFALFA FOR HIGH YIELD AND QUALITY FORAGE

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Abstract

Biological nitrogen fixation and assimilation of CO_2 can be indirectly monitored in alfalfa (*Medicago sativa* L.) through the variation of the stable N and C isotopes in the plant (Δ values). Such measurements result in a simple, non-destructive and reliable method for identification of alfalfa cultivars of high yield and nutritional quality. A program for selection of 12 alfalfa cultivars under field and controlled conditions (lysimeters) included determination of biomass and N accumulation. Under controlled conditions yields ranged from 1.77 to 2.47 ton/ha/month (Pioneer 5312 and WL-605) and N2-fixed varied from 77.43 to 84.01% for total N (Brazilian Creoule and Victoria). ΔC varied from 21.53 to 21.94 (Pioneer 5312 and Brazilian Creole). Significant correlations between yield and ΔC (0.57*) and N from fixation (0.77*) showed the potential of using Δ values in alfalfa germplasm evaluation for high biomass yield and atmospheric N accumulation.

Keywords: Alfalfa, carbon isotopic discrimination, biological nitrogen fixation

Introduction

Plant properties that indicate its yield potential and quality are associated with common agronomic traits such as dry matter, digestibility and protein content. In alfalfa (*Medicago sativa* L.) the biologic nitrogen fixation, measured via the isotopic discrimination of nitrogen, allows the identification of more efficient plants for the formation of nitrogen compounds, which influence the biomass production (Oliveira, 1999). Likewise, the photosynthetic capacity, measured via the isotopic discrimination of carbon, allows the identification of more efficient plant for the maximization of the stomatal opening, which regulates the biomass production (Farquhar, *et al*. 1989; Bouton, 1997).

A selective program that studied twelve different cultivars of alfalfa, including dorments and non dorments varieties, was initiated in 1997 at the Superior School of Agronomy "Luiz de Queiróz", Piracicaba, SP, Brazil. This program was based upon analyses of the plant yield potential, plant quality and environmental adaptation of the varieties of interest in the geographic region of Piracicaba.

Material and Methods

An experiment was initiated, under controlled conditions, in lysimeters located at the Center of Nuclear Energy for Agriculture (CENA)/USP, Piracicaba, SP., Brazil. This study was randomly designed, with 4 replications placed within the 12 lysimeters of 3 m^2 each and 1.5 m of depth, filled with red-yellow latossol soil. The experiment was monitored during 7 months performing 6 harvests. The effects of water factors, such as rainfed and irrigated, and the cultivars (Brazilian Creoule, Chilean Creoule, Victoria, UC-Cíbola, Moapa-69, WL-516, WL-605, SW-14, Mecca, Mecca-II, Pioneer-5312 and Pioneer XAI32) were evaluated by the number of stems, dry matter and stem/leaf ratio, after drying them at 60 °C for 72 h. The total nitrogen obtained after sulphuric digestion and its quantification were evaluated by colorimetric assay. The isotopic discriminations of carbon and nitrogen derived from atmosphere and obtained through the isotopic composition were evaluated by mass spectrometry type ANCA-IRMS. Complementary irrigation was performed when necessary according to the mercury tensiometer.

Results and Discussion

Under controlled conditions significant variations ($p<0.05$) were observed for all agronomic and physiological traits for 12 alfalfa cultivars (Table 1). Values of dry matter ranged from 1.77 to 2.47 ton/ha/month (Pioneer 5312 and WL-605). The number of stems varied from 516.2 to 676.8/m^2 (WL-516 and Victoria) and the ratio stem to leaves was between 0.78 to 1.00 (Victoria and WL-605). Total N varied from 3.07 to 3.50% (WL-605 and Victoria), N_2-fixed ranged from 77.43 to 84.01% (Brazilian Creoule and Victoria) and ΔC varied from 21.53 to 21.94 (Pioneer 5312 and Brazilian Creole).

The severity of the stress undergone by the rainf treatments was indicated by significant variation compared with the irrigated treatments (Table 1). Under rainfed conditions only total nitrogen in dry matter and the ratio stem to leaf were significantly positive. This is supported by the reduction of the carbon gain via stems growth and biomass accumulation. Similar results were documented for alfalfa (Ray *et al.*, 1998) and for common bean (White *et al*., 1990).

Selection for low ΔC has been proposed as a method to improve WUE (water use efficiency) in clones or populations of alfalfa (Johnson *et al*., 1995, Ray *et al*., 1998). Low ΔC has been associated with low yield and N_2-fixed among cultivars (Knight *et al*., 1993, Boutton, 1997).

Significant correlations between yield and ΔC (0.57*) and N from fixation (0.77*) showed the potential of using Δ values in alfalfa germplasm when evaluating high biomass yield and atmospheric N accumulation. The results indicate that differences in stomatal conductance, photosynthetic capacity and/or capacity of biological nitrogen fixation exist among the 12 alfalfa cultivars, and the cultivars of high biomass growth tended to show high ΔC values and N-fixed.

Preliminary data observed from field experiment show similar results to those observed under controlled conditions. These experiments have been conducted for 22 months and they will end in May/2000.

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Table 1 – Agronomic and physiological traits for 12 alfalfa cultivars under rainfed and irrigated conditions. Mean of 4 replications and 6 cuts.

	Lysimeter Data					
	Dry Matter (t/ha)	Number of stems (m^2)	Stem/leave ratio	N total (%)	N^2-fixed (%)	Carbon isotopic discr. ($\Delta^{13}C_{PDB}$)
Cultivar						
Crioula Brasil	1,98	666,80	0,84	3,46	77,43	21,82
Crioula Chile	2,32	588,30	0,82	3,25	82,77	21,94
Victoria	2,00	676,80	0,93	3,50	84,01	21,74
UC-Cíbola	2,39	597,60	0,92	3,20	81,38	21,63
Moapa-69	1,98	522,30	0,91	3,20	80,99	21,75
Wl-516	2,05	516,20	0,88	3,17	80,84	21,70
WL-605	2,47	613,70	0,88	3,07	82,74	21,65
SW-14	2,29	619,50	0,87	3,23	80,70	21,79
Mecca	2,38	598,30	0,92	3,23	80,51	21,89
Mecca-II	2,20	613,70	0,83	3,18	81,33	21,82
Pioneer-5312	1,77	624,50	0,91	3,43	79,78	21,53
PioneerXAI32	2,13	577,30	0,93	3,30	82,42	21,74
DMS(0.05)	0,29	88,00	0,07	0,22	1,74	0,34
NS	0,00001	0,00001	0,0001	0,00015	0,00001	0,00827
Water						
Irrigation	2,45	643,00	0,94	3,21	85,15	22,00
Rain	1,88	559,50	0,84	3,33	69,72	21,50
DMS(0.05)	0,80	7,14	0,02	0,05	0,40	0,08
NS	0,00001	0,00001	0,0001	0,00001	0.0001	0,00001
Average	2,16	60,13	0.89	3,27	81,24	21,75
C.V.	8,05	8,66	4,80	3,37	0,84	0,78