Biochemical and Isoenzyme Analysis of Seven Pennisetum Purpureum (schum.) Cultivars

E E. Bach
Instituto Biologico

V B.G. Alacantara
Instituto de Zootecnia

P B. Alcntara
Instituto de Zootecnia

E A. Veasey
Instituto de Zootecnia

BIOCHEMICAL AND ISOENZYME ANALYSIS OF SEVEN PENNISETUM PURPUREUM (SCHUM.) CULTIVARS

E.E. Bach[1], V. B. G. Alcântara[2], P. B. Alc,ntara2* and E. A. Veasey[2]
[1]Instituto Biológico, C. P. 7119, 01064-970 - S. Paulo/SP, Brazil
[2]Instituto de Zootecnia (Animal Science and Pasture Institute), C.P. 60, Nova Odessa/SP, Brazil
*CNPq's scholarship

ABSTRACT

This study characterized seven *Pennisetum purpureum* cultivars, namely cv. Anão, Bajra, Cameroon, Guaçu, Roxo, Taiwan A-144 and Uruckwami, through biochemical analysis, including protein, glucose and fructose contents, and polyacrylamide gel electrophoresis using the esterase system, by sampling 30, 60, 90, 120 and 150 day-old leaves. Cultivar Taiwan A-144 presented the highest number of nodes per stem and percentage of emerging buds. Protein concentration decreased gradually after 60 days for all cultivars, except for Anão. Cultivar Guaçu presented the highest level of glucose in 90 day old plants, whereas Cameroon presented the highest levels at 120 and 150 days. The esterase band patterns changed with plant age for all cultivars, showing a tendency to increase the number of bands with time. The best age for discriminating between esterase bands of *P. purpureum* cultivars was at 120 days, when most variation could be seen.

KEYWORDS

Electrophoresis; reducing sugars; proteins; elephant grass; *Pennisetum purpureum*

INTRODUCTION

Elephant grass (*Pennisetum purpureum*) is one of the most important grasses in the world's tropic and subtropic regions (Tcacenco & Botrel, 1990). It is valued for its competitive ability and persistence, palatability and good herbage quality with high yields amounting to 300t. green matter/ha/y (Bogdan, 1977).

Many ecotypes have been selected however, little is known about the real difference between these cultivars, possibly because many have been introduced to different places with different names with no documentation of the original identities.

The discrimination of cultivars is almost always a difficult task, due to phenotypical similarities that may exist among them, which could be overcome by biochemical analysis, such as protein and enzyme. The esterase system is the most conclusive isoenzyme system for varietal identification due to the complexity and quality of its patterns (Gottlieb, 1981).

This paper reports on the content of soluble proteins, carbohydrates and esterase isoenzyme of seven *Pennisetum purpureum* cultivars, some of them widely used in Brazil. Knowledge of these compounds may help to explain important aspects such a taxonomic identification and also the relationships with microorganisms.

METHODS

Elephant grass cuttings of seven cultivars (Guaçu, Bajra, Anão, Cameroon, Roxo, Taiwan A-144 and Uruckwami) were planted in plastic bags containing fertilised soil. This material was maintained in the greenhouse for 30 to 150 days. The percentage of bud emergence was recorded at 8, 12, 17, 20 and 22 days after planting. Samples from 30, 60, 90, 120 and 150 day-old plants, marked on the second and third leaves from the apex of 30 day-old plants, were taken using two different methods: A - ethanolic extraction; B - buffer extraction.

For ethanolic extraction fresh cut small pieces of leaves without the midrib were dropped into 100 ml boiling ethanol (80%) for 5 minutes. After cooling, the extracted material was homogenised and filtered. Glucose and fructose were analysed by the method of Somogyi (1945).

For proteins, three grams of fresh cut small pieces of leaves without the midrib, were homogenised with 30ml phosphate buffer (0.1M pH 6.5), containing NaCl, mercaptoethanol, and sucrose. Total protein was determined by the method of Lowry *et al*. (1951) and isoenzymes were analysed by electrophoresis, using the esterase system prepared by the method of Stegemann *et al.* (1987). Sample solutions, standardised to 300 ug equivalent BSA/ml, were located on the gel. The average relative mobility (Rm) value of three gels was recorded for each extract. Gels were scanned in a densitometer and zymograms were prepared to compare the band patterns.

RESULTS

Cameroon presented the lowest number of nodes per stem and Taiwan A-144 the highest at 141 days after cutting. Cultivar Taiwan A-144 was the fastest to emerge, whereas Roxo, Guaçu and Uruckwami were slower to emerge, although Roxo showed a good recovery after the 12th day.

The protein contents gradually decreased after 60 days for all cultivars, except for Anão, which showed the highest levels of protein at 90 and 120 days. Cultivar Guaçu also presented a high level of protein at 90 days, decreasing gradually afterwards.

All cultivars showed a low level of fructose up to 60 days, but increasing after 90 days. Cultivar Roxo presented significantly higher levels of fructose than all the other cultivars at 120 and 150 days. Cultivars Guaçu and Cameroon showed higher levels of glucose at 90 days, while an increase at 120 day-old leaves. However, at 150 days the glucose concentration decreased for all cultivars.

Esterase isoensymes were assayed from leaves of 30 to 150 day-old leaves. Band patterns changed with plant age for all cultivars. The number of bands at 30 and 60 days were the same and have a slightly differences in the varieties. The number of bands increased at 90 days and was more intense at 120 and 150 day-old leaves.

The best leaf age for *P. purpureum* cultivar discrimination, using the esterase system, was considered to be at 120 day-old leaves, when most variation was observed for each cultivar.

DISCUSSION

Proteins and reducing sugars in leaves of *Pennisetum purpureum* were affected by the leaf age and cultivar. In the present study, protein content decreased with leaf age, while the reducing sugars (fructose and glucose separately), showed different results. Fructose increased while glucose decreased after 120 days for all cultivars (according to Shree and Reddy, 1986).

Electrophoresis of esterase indicated that some isoenzymes were peculiar to young leaves (30-60 days), and others to older plants (90, 120 and 150 days). This is in the accordance to Johnson (1974). In the present study, a better resolution in electrophoresis was obtained in adult leaves, demonstrating the existence of alteration in the isoenzymatic patterns during plant development.

In the morphological studies, cultivar Taiwan A-144 presented the highest number of nodes per stem and also fastest to emerge, according to Alcântara *et al*. (1980). However, that cultivar presented in our study the lowest concentration of proteins, being surpassed by all the other cultivars.

This study demonstrated that the isoenzymatic characterization is an important complement to morphological and biochemical data for differentiating cultivars.

REFERENCES

Alcantara, P.B, V.B.G.and J.E. Almeida. 1980. Estudo de vinte e cinco prováveis variedades de capim-elefante (*Pennisetum purpureum* Schum.). Boletim de Indústria Animal, Nova Odessa, **37:** 279-302.

Bogdan, A.V. 1977. Tropical Pasture and Fodder Plants (Grasses and Legumes). New York, Longman Inc., 475p. (Tropical Agricultural Series).

Gottlieb, L.D. 1981. Electrophoreic evidence and plant populations. Progress in Phytochemistry, London, **7:** 1-46.

Johnson, G.B.1974. Enzyme polymorphism and metabolism. Science, Washington, **184:** 28-37.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randell. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, Baltimore, **193:** 265-275.

Shree, M.P. and C.N. Reddy. 1986. Effect of helminthosporiose infection on certain biochemical constituents in the resistant and susceptible varieties of Sorghum. Indian Journal of Plant Pathology, New Delhi, **4:** 46-52.

Somogyi, M. 1945. A new reagent for the determination of sugars. Journal of Biological Chemistry, Baltimore, **160:**. 61-68.

Stegenmann, H., W. Burgermeister and A.A. Shah. 1987 Gel-elektrophorese und isoelektrische-fokussierung. Bba, Braunschweig.

Toacenco, F.A. and M.A. Botrel. 1990. Identificação e avaliação de acessos e cultivares de capim elefante. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 1990, Coronel Pacheco. Anais, Coronel Pacheco: Sociedade Brasileira de Zootecnia, pp. 1-22.

Figure 1
Protein concentrations in the leaves of Pennisetum purpureum cultivars: U-Uruckwami, A-An,,o, G-GuaÁu, C-Cameroon, R-Roxo, B-Bajra, T-Taiwan A-144. Means between cultivars and between leaf ages are significantly different (P,0.05%), according to the Studentís t-test.

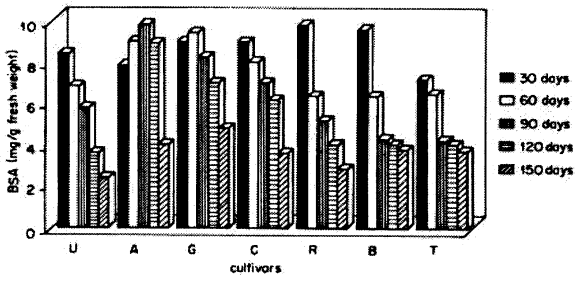

Figure 2
Estarase zymograms of foliar extract of *Pennisetum purpureum* cultivars: G-Guaçu, C-Cameroon, U-Uruckwami, B-Bajra, T-Taiwan A-144, A-Anão, R-Roxo, of 30, 60, 90, 120 and 150 day-old leaves.