In Vitro Propagation of *Pennisetum purpureum* Schum.

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IN VITRO PROPAGATION OF *Pennisetum purpureum* Schum.

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Abstract

A protocol is described for rapid multiplication of elephantgrass (*Pennisetum purpureum* Schum.) through shoot tip culture. The plant growth medium consisted of basal medium of Murashige and Skoog (MS) and vitamins Wood Plant Medium (WPM). The medium was supplemented with 0.00; 4.44; 8.88; 13.32 and 17.76 μM of benzylaminopurine (BAP). The elephantgrass was micropropagated by axillary shoot proliferations. Maximum propagule proliferation occurred on Murashige and Skoog (MS) medium enriched with 4.4 μM benzylaminopurine (BAP), resulting an average of 4.0 shoots per explant from cultivar Mineiro and 2.19 from cultivar Pioneiro. The best height and root plantlets were obtained with medium without growth regulator after 28 days.

Keywords: *Pennisetum purpureum*, in vitro propagation, growth regulator

Introduction

The virus status of explants for in vitro culture is often unknown, but may play an important role in micropropagation. Inconsistent results and death of plant materials in tissue culture experiments might be due to undetected virus infection. Tissue culture provides an alternative method to mass propagation plant in a short time, with vigor and without diseases (Ferreira et al. 1998). Also in vitro techniques have proven extremely useful in the conservation of rare and endangered species that are difficult to propagate by conventional methods.

Zanette et al. (1988) induced shoot proliferation from elephantgrass using meristem culture after 35 days. Also, induction of embryogenic callus was obtained on leaf explant of elephantgrass by Passos and Katterman (1994).

This investigation was undertaken to determine cytokinin level effect on root and shoot proliferation to develop a method to micropropagate elephantgrass free of diseases.

Material and Methods

Shoot tips of elephantgrass were surface disinfected with 70% ethanol (5 s), rinsed with sterile distilled water, gently shaken in 1.5% sodium hypochlorite (15 minutes), and finally rinsed three times with sterile distilled water. After establishment of the shoot tip, the axillary shoot was used for shoot proliferation.

Explants were cultured in 150 x 25 mm test tubes, containing 15 ml of medium solidified with agar ($7g.l^{-1}$). Media were adjusted to pH 5.7 (before autoclaving for 20 minutes). Cultures were incubated under a 16 h photoperiod (2000 lux) at 26±1°C.

Individual shoots (30 mm long) were cultured on a basal medium of MS (Murashige and Skoog, 1962) and vitamins WPM medium (Lloyd and McCown, 1980), supplemented with 0.00;

4.44; 8.88; 13.32 and 17.76 μM of benzylaminopurine (BAP). Shoot proliferation was assessed after 28 days by counting the number of induced shoots per explant. Average height and root plantlets numbers were obtained per explant.

Results and Discussion

Shoot Proliferation

The number of shoots produced per explant were significantly affected ($P<0.05$) by BAP (benzylaminopurine) concentration (Table 1). Shoot tip failed to stimulate shoot formation on cytokinin-free medium. In contrast, the shoot tip explant showed an initial swelling at the cut end in cytokinin-rich MS basal medium. The number of new emerging shoots varied according to the concentration of cytokinin used. Maximum number of shoots per explant were obtained with 4.44 μM of BAP. Four shoots per explant were obtained at the end of each passage (28 days) by the cultivar Mineiro, whereas the maximum number of shoots per explant for the cultivar Pioneiro was 2.19.

Plantlet Height

The height of plantlets was significantly ($P<0.05$) affected by growth regulator (Table 1). The growth regulator (BAP) had a detrimental effect on plantlet height and rooting. Maximum height was obtained for the medium without growth regulator. The plantlets reached heights of 4.12 and 4.91 cm for cultivar Mineiro and cultivar Pioneiro, respectively, at the end of 28 days.

Root Number

The number of root formed per explant was significantly ($P<0.05$) affected by growth regulator (Table 1). The microshoots obtained through in vitro shoot multiplication were best rooted in MS basal medium free of growth regulator.. After 28 days it was observed 2.56 and 2.70

roots per explant for cultivar Mineiro and cultivar Pioneiro, respectively. The rooted plants were transferred to subtrate, acclimatized in a growth chamber. These plantlets showed a survival rate of 98 to 100%.

This study highlights a micropropagation protocol for elephantgrass through shoot tip culture. Best shoot proliferation was obtained with 4.44 μM of BAP (4.0 and 2.19 shoots per explant) with the cultivar Mineiro and cultivar Pioneiro, respectively. The best plantlet heights were obtained without growth regulator, 4.12 and 4.91 cm for cultivar Mineiro and cultivar Pioneiro, respectively. Also, the best rooting was obtained with the medium without growth regulator for both cultivars (2.56 and 2.70 roots per explant) with the cultivar Mineiro and Pioneiro, respectively.

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Table 1 – Growth characteristics of elephant grass according to benzylaminopurine (BAP) concentrations and cultivars.

BAP (μM)	Cultivar					
	Mineiro			Pioneiro		
	Shoots proliferation (n^0)	Roots number (n^0)	Plantlet heights (cm)	Shoots proliferation (n^0)	Roots number (n^0)	Plantlet heights (cm)
0.0	0.16A c	2.56A a	4.12B a	1.13A b	2.70A a	4.91A a
4.44	4.00A a	2.10A ab	3.79A ab	2.19B a	1.46A b	3.23B b
8.88	3.73A ab	2.26A ab	3.68A ab	2.03B a	1.16B b	3.23B b
13.32	2.76A b	1.33A b	3.52A b	1.46B a	0.79A b	3.11B b
17.76	2.99A ab	1.42A b	3.43A b	1.70B a	0.89A b	3.23A b

Means followed by different letters, small letters in the columns and capital letters in the rows, are different ($P<0.05$) by Tukey test.