

Isolation of root endophytic bacteria in elephant grass (*Pennisetum purpureum*) cultivars

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Introduction

Elephant grass (*Pennisetum purpureum*) is one of the most productive warm-season grasses. Farmers utilize elephant grass in different forms, such as cut-and-carry, grazing, conserved forage (silage, hay), and as an energy source (Lira et al. 2010). Nitrogen (N) is an essential element for plant growth and development and is usually a limiting factor for forage production in the tropics (Boddey et al. 2004). Biological N fixation (BNF) may occur in warm-season grasses by their association with diazotrophic bacteria. These bacteria colonize different niches in the host plant: Endophytic bacteria form colonies inside the plant tissue, whereas epiphytic bacteria colonize external surfaces of plants (Compant et al. 2010). Both types of bacteria may benefit host plants (Badri et al. 2009). This study evaluated endophytic diazotrophic bacteria density associated with the roots of different elephant grass cultivars using 2 N-free growth media, at different times after inoculation.

Materials and Methods

Root samples from 3 elephant grass cultivars (cvv. Elefante B, Venezuela and Pioneiro) were collected in October 2012. These cultivars had been growing for 3 years under cutting management without N fertilization, at the Agronomic Research Institute of Pernambuco (IPA), Itambé, Pernambuco, Brazil. Root endophytic bacteria were isolated following the methodology of

Döbereiner et al. (1995) and Kuklinsky-Sobral et al. (2004). Inoculations were performed using a serial dilution method (10^{-3} , 10^{-4} and 10^{-5}) in a saline-phosphate buffering solution. Subsequent inoculation was replicated 5 times using the semi-solid media, NFb and JMV (Baldani et al. 2000), adding to each sample 50 µg/mL of Thiophanate Methyl, a commercial fungicide (Cercobin 700®). The semi-solid N-free media, NFb (neutral pH) and JMV (acid pH), are commonly used for enrichment and isolation of bacteria (Döbereiner et al. 1995; Videira et al. 2007). Samples were incubated thereafter at 28 °C. Bacterial growth inside the media was evaluated 5, 8, 10 and 12 days after inoculation. The most probable number (MPN) of diazotrophic bacteria per gram of sample was determined using the McCrady table as described by Döbereiner et al. (1995). Bacterial population densities were expressed at the log₁₀ basis and submitted to ANOVA using the statistical software package SISVAR 5.3®; means were compared by Tukey test ($P < 0.05$).

Results and Discussion

For the JMV medium, MPN of microbial cells per gram of fresh vegetal tissue (FVT), regardless of time since inoculation, ranged from 5.50×10^4 to 1.57×10^5 ; from 3.23×10^4 to 4.5×10^4 ; and from 6.17×10^5 to 7.00×10^5 for Elefante B, Venezuela and Pioneiro, respectively. For the NFb medium, MPN per gram of FVT ranged from 4.70×10^4 to 6.60×10^4 ; from 7.00×10^3 to 2.53×10^4 ; and from 5.76×10^5 to 6.46×10^5 for Elefante B, Venezuela and Pioneiro, respectively. No significant difference was observed ($P > 0.05$) for MPN per gram of fresh vegetal tissue between media (Table 1).

Time after inoculation (5, 8, 10 and 12 days), cultivar and isolation medium had no effect on microbial density.

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Table 1. Root endophytic diazotrophic bacteria density (log MPN cells/g FVT) in elephant grass (*Pennisetum purpureum*) cultivars in 2 N-free media (JMV and NFb) and at varying times since inoculation.

Elephant grass cultivars	Isolation medium	Reading days after inoculation			
		Day 5	Day 8	Day 10	Day 12
Elefante B	JMV	4.63 Aa ¹	4.96 Aa	5.19 Aa	4.71 Aa
	NFb	4.43 Aa	4.47 Aa	4.75 Aa	4.50 Aa
Venezuela	JMV	4.47 Aa	4.45 Aa	4.59 Aa	4.44 Aa
	NFb	3.89 Aa	4.00 Aa	4.15 Aa	3.83 Ab
Pioneiro	JMV	5.00 Aa	5.00 Aa	5.45 Aa	5.45 Aa
	NFb	5.04 Aa	5.14 Aa	5.18 Aa	5.18 Aa

¹Means followed by the same letter, upper case within rows and lower case within columns, do not differ (P>0.05) by Tukey test.

Conclusions

High numbers of root endophytic diazotrophic bacteria are associated with elephant grass, with no differences between cultivars. The benefit of this association to the plants is the subject of other studies.

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