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

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Introduction

Elephant grass (*Pennisetum purpureum* Schum.) is one of the most productive warm-season grasses. Farmers utilize elephant grass in different forms, such as cut-and-carry operations, 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 it 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 plant external surfaces (Compant *et al.* 2010). Both types of bacteria may benefit host plants (Badri *et al.* 2009). This study evaluated endophytic diazotrophic bacteria density associated to the roots of different elephant grass cultivars (cvs. 'Elefante B', 'Venezuela', and 'Pioneiro') using two N-free growth media, at different evaluation periods.

Materials and Methods

Root samples from three elephant grass cultivars (cvs. 'Elefante B', 'Venezuela', and 'Pioneiro') were collected in October 2012. Prior to sampling, these cultivars were growing for three years under cut management without N fertilization, at the Agronomic Research Institute of Pernambuco (IPA), located in Itambé, Pernambuco, Brazil. Root endophytical bacteria were isolated according to Döbereiner *et al.* (1995) and Kuklinsky-Sobral *et al.* (2004). Inoculations were performed using a serial dilution method (10^{-3}, 10^{-4} e 10^{-5}) in a saline-phosphate buffering solution. Subsequent inoculation was replicated five times using solid media NFb and JMV (Baldani *et al.* 2000), adding in each sample 50 µg/mL of Thiophanate Methyl, a comercial fungicide (Cercobin 700®). Samples were incubated thereafter at 28°C. Bacteria 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 McCrady table, according to Döbereiner *et al.* (1995). Bacterial population densities were expressed at the $\log_{10}$ 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, the MPN of microbial cells per gram of fresh vegetal tissue (FVT), regardless of evaluation timing, 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 FVT between media (Table 1).

Table 1. Root endophytic diazotrophic bacteria density (log MPN cells/g fresh vegetal tissue) in elephant grass (*Pennisetum purpureum* Schum.) cultivars in two N-free media (JMV and NFb) and inoculation periods

Elephant grass cultivars (*Pennisetum purpureum*)	Isolation medium	Reading days after isolation			
		Day 5	Day 8	Day 10	Day 12
'Elefante B'	JMV	4.63 Aa	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

Means followed by the same letter, capital letters within the column and small letters within the row, do not differ ($P > 0.05$) by Tukey test.

Reading time (5, 8, 10, and 12 days) did not affect microbial density. Elephant grass cultivars did not differ at the different inoculation times, for both media.

Conclusions

Elephant grass cultivars presented high association with root endophytic diazotrophic bacteria. Growth media (JMV and NFb) and inoculation time (5, 8, 10, and 12 days) did not affect microbial population density.

References

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