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Potential use of endophytic bacteria to promote the plant growth of micropropagated banana cultivar Prata Anã

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Endophytic bacteria isolated from shoot tip cultures of banana 'Tropical' and 'Galil 18' were identified at the genera and species levels by means of the profile of fatty acids test through gas chromatography and the potential of plant growth promotion of micropropagated 'Prata Anã' banana plantlets was evaluated. A completely randomized experimental design in a factorial scheme 2 × 9, with two immersion periods (30 and 60 min), eight isolates (endophytic bacteria) plus control (saline solution; 0.85% sodium chloride) with seven replications was used. The plantlets were cultured under greenhouse conditions and the evaluations were performed at 24 (DAP_1) and 48 (DAP_2) days after planting. The isolates of endophytic bacteria affected significantly pseudostem height, number of leaves and pseudostem diameter. Five isolates: 03, 05, 06, 07 and 08 showed percentages of increment index over 20% for pseudostem height. Among them, the isolate 07, *Klebsiella pneumoniae pneumoniae* showed the highest increment indices: 27.48, 13.68 and 13.55% for pseudostem height, number of leaves and pseudostem diameter, respectively. The results show the potential use of *K. pneumoniae pneumoniae* (Isolate 07) for growth promotion in micropropagated 'Prata Anã' banana plantlets.

Key words: *Musa* sp., *Klebsiella pneumoniae pneumoniae*, plant growth promoting bacteria.

INTRODUCTION

Micropropagation is a successful technique for mass production; the plantlets present the advantages of affording genetic and phytosanitary quality, in addition to considerable increases in the number of plants within a short period of time (Souza et al., 2000). However, micropropagated plants presents some inconveniences such as less vigorous and require more attention and handling care. This occurs as a consequence of the sterile and aseptic system in which the plantlets were produced. Plantlets derived from tissue culture are completely free from beneficial microorganisms and present a defense mechanism that is not activated (Dubois et al., 2006). Numerous studies have demonstrated the great potential of endophytic microorganisms in agricultural production: biological fixation of nitrogen, resistance to abiotic stress (Teixeira et al., 2007),biocontrol

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Table 1. Nutrient content resulting from the chemical analyses of Bioplant® substrate used in the experiment, Janaúba, Minas Gerais, Brazil.

Substrate	pH[1]	P[2]	K[2]	Ca[3]	Mg[3]	Al[3]	Al+H[4]	SB	Cu[2]	Zn[2]	OM[5]
		--mg/dm^3--		cmolc/dm^3					-mg/dm^3-		dag/kg
Bioplant®	4.9	549.7	1306	11.2	4.3	0.1	7.7	19.5	1.9	18.3	19.7

Analyses performed by Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG, Unidade Regional Norte de Minas, Nova Porteirinha – MG. [1]pH in water; [2]Extractor: Mehlich-1; [3]Extractor: KCl 1 mol/L; [4]pH SMP; [5]Colorimetry. SB, Sum of bases; OM, organic matter.

of diseases (Romeiro et al., 2007), and production of plant growth regulators (Azevedo et al., 2000). The genera of greater occurrence in different species including bananas are: *Pseudomonas*, *Enterobacter*, *Burkholderia* (Thomas et al., 2008), *Bacillus* (Teixeira et al., 2007; Jie et al., 2009; Souza et al., 2013) and *Klebsiella* (Martínez et al., 2003).

The successful colonization of Plant Growth-Promoting Bacteria (PGPB) is essential for increasing the natural defense system of the plant and plant growth promotion (Dubois et al., 2006; zum Felde et al., 2009). According to Nowak and Pruski (2002), the endophytic microbial inocula are used as propagule priming agents, both as *in vitro* culture and on transplanting phase. Jie et al. (2009) indicated that the re-introduction of naturally-occurring endophytes into tissue culture banana plantlets can improve the disease suppression, plant growth and yield. A study conducted by Jaizme-Vega et al. (2004) also showed positive results of bacterial application on the first developmental stages of two micropropagated bananas cultivars. Foliar mineral contents were significantly increased only in Grand Naine cultivar and significant increments were observed in the growth of aerial part, root and total fresh weight of the both cultivars.

The objective of the present study was to evaluate the artificial inoculation of banana plants with eight endophytic bacteria isolates on plant growth parameters in a greenhouse experiment.

MATERIALS AND METHODS

Plant materials and inoculum preparation

Micropropagated banana plantlets from the cultivar Prata Anã were used in the present study. The endophytic bacteria were isolated from roots of bananas cultivars Tropical and Galil 18, and were identified at the genera and species levels by means of the profile of fatty acids test through gas chromatography (Ganen et al., 2008). The isolates were inoculated on a medium surface containing potato, dextrose and agar (HKM CHINA, China) (PDA), in Petri dishes for a period of 48 h at a temperature of 27°C. A single colony was transferred to 500 ml flasks containing Tryptic Soy Broth (TSB) and the cultures were incubated at 28°C for 48 h with 100 rpm shaking. The concentration of bacterial cells in the suspension was determined by reading the absorbance at a wavelength (λ) of 540 nm on a spectrophotometer (approximately 10^8cfu/ml^{-1}) and each culture was adjusted to have an optical density (OD) equal to 1.0 ABS. The eight selected isolates and the control (saline solution: 0.85% sodium chloride) are described in Table 2.

Artificial inoculation

For each treatment, 100 ml of the bacterial suspensions and the saline solution were used for immersion of the banana roots. Two root immersion periods were evaluated at 30 and 60 min. The control plantlets were immersed in saline solution (0.85% sodium chloride) during the same immersion periods. After the immersion periods, the plantlets were planted in plastic tubes (50 cm^3) containing the commercial substrate BIOPLANT® (Ponte Nova, Brazil) (Table 1). Subsequently, the plantlets were moved to the acclimatization phase in a greenhouse, with a temperature ranging from 25 to 35°C. During the evaluation period no fertilizers were used.

Assessment of variables

The evaluations were performed at 24 (DAP_1) and 48 (DAP_2) days after planting. The following characteristics were evaluated: pseudostem height (cm), number of leaves and pseudostem diameter (cm). The increment index (II), expressed as a percentage, was estimated for all traits by using the formula proposed by Edgington et al. (1971):

$$II = [(TR - T)/ T]100$$

Where, TR = value obtained for the treatment tested at DAP_2, and T = value obtained for the treatment tested at DAP_1.

Experimental design and statistical analysis

A completely randomized experimental design in a factorial scheme 2 × 9, with two immersion periods, nine treatments and seven replications was used. The data were submitted to analysis of variance (ANOVA) using the SISVAR statistical software package (Ferreira, 2008). Means separation among treatments was performed by Scott-Knott test at the 1% probability level based on the F-test of ANOVA.

RESULTS AND DISCUSSION

There were no significant differences ($p< 0.01$) for immersion periods and the interaction between isolates and immersion periods for all traits were evaluated (data

Table 2. Bacteria identification, pseudostem height (cm), number of leaves, diameter of the pseudostem (cm) and increment index (II) in the 'Prata Anã' banana plantlets at 24 (DAP1) and 48 (DAP2) days after planting submitted to different endophytic bacteria isolates, Janaúba, Brazil.

Bacteria	Treatment	Pseudostem height (cm)			Number of leaves			Pseudostem diameter (cm)		
		DAP 1	DAP 2	II (%)	DAP1	DAP2	II (%)	DAP1	DAP 2	II (%)
-	Control	3.02[a]	3.17[a]	4.97	5.43[a]	4.93[a]	-9.20	0.67[a]	0.74[a]	10.44
Peudomonas hutiensis	Isolate 01	3.53[a]	3.57[a]	1.13	5.36[a]	5.07[a]	-5.41	0.72[a]	0.78[a]	8.33
Enterobacter cloacae	Isolate 02	2.22[b]	2.52[b]	13.51	4.78[b]	4.78[a]	0	0.66[b]	0.68[a]	3.03
Klebsiella pneumoniae pneumoniae	Isolate 03	2.38[b]	2.83[b]	20.16	4.57[b]	4.86[a]	6.34	0.60[b]	0.67[a]	11.66
Paenibacillus polymyxa	Isolate 04	2.58[b]	2.92[b]	13.18	4.28[b]	4.43[a]	3.50	0.63[b]	0.67[a]	6.34
Klebsiella pneumoniae pneumoniae	Isolate 05	2.54[b]	3.12[a]	22.83	4.71[b]	5.07[a]	7.64	0.62[b]	0.68[a]	9.67
Klebsiella pneumoniae pneumoniae	Isolate 06	2.60[b]	3.16[a]	21.53	4.77[b]	4.78[a]	0.21	0.63[b]	0.68[a]	7.93
Klebsiella pneumoniae pneumoniae	Isolate 07	2.62[b]	3.34[a]	27.48	4.31[b]	4.90[a]	13.68	0.59[b]	0.67[a]	13.55
Klebsiella pneumoniae pneumoniae	Isolate 08	2.61[b]	3.22[a]	23.37	4.71[b]	4.78[a]	1.48	0.59[b]	0.66[a]	11.86
CV (%)		25.49	20.67	-	19.29	16.52	-	14.48	14.89	-

Averages followed by different letters in the columns differ amongst themselves, according to the Scott-Knott test, at a 1% probability.

not shown). In general, the isolates of endophytic bacteria evaluated in the present study were capable for promoting growth in banana plantlets cultivar Prata Anã. The results show that the endophytic bacteria affected significantly pseudostem height, number of leaves and pseudostem diameter. At 48 days after planting, the plantlets inoculated with isolate 01 (*Pseudomonas huttiensis*) exhibited the greatest pseudostem height with an average of 3.57 cm. However, the increment index was very low, 1.13% (Table 2). The highest percentage of increment index (27.48%) was observed in plantlets inoculated with isolate 07 (*Klebsiella pneumoniae pneumoniae*). The isolates 03, 05, 06 and 08 also promoted percentages of increment over 20%. These isolates were also classified as *K*. *pneumoniae pneumoniae* (Table 2). The isolate 01 and the control treatment promoted negative increment indices (-9.20 and -5.41%, respectively), for number of leaves per plantlet. Once again, the greatest increment in number of leaves was achieved with the inoculation of isolate 07, *K*. *pneumoniae pneumoniae*. This isolate promoted an increment of 13.68% for this trait (Table 2).

The plantlets inoculated with isolate 01 showed the highest average for pseudostem diameter, 0.78 cm at 48 days after planting. Otherwise, the increment index was lower than 10% (Table 2). Three isolates (03, 07 and 08) presented increment indices over 10% for pseudostem diameter. The isolate 07 showed the largest increment index of 13.55% (Table 2). The micropropagated banana plantlets of the cultivar Prata Anã inoculated with the endophytic bacteria exhibited different responses to the variables analyzed, indicating the effect of various isolates in the growth and development of the plantlets. The observed variations in the development of the plantlets can be attributed to multiple factors: stress experienced during the acclimatization phase, association capacity of the isolate, variations in the balance of the endophytic bacterial population, as well as the evaluation period (Whipps, 2001). The mechanism of growth improvement was beyond the objective of our investigation, but the increment observed in inoculated plantlets is probably associated to the production of plant growth regulators. Jie et al. (2009) evaluating the artificial inoculation of banana tissue culture plantlets with indigenous endophytes showed that the re-introduction promoted increments in pseudostem height, pseudostem diameter and leaf area. The authors also suggested that the enhancement observed in growth characteristics is probably associated with capacity of production of plant growth regulators. Similar results were obtained by Ting et al. (2008), the authors showed the great potential of a bacterial isolate, *Serratia* sp. as growth promoting in banana plantlets.

Various studies have showed the capacity of production of indole-3-acetic acid (IAA) by the

endophytic bacteria. Yuan et al. (2013) evaluating the ability of *Bacillus amyloliquefaciens* to promote the growth of banana plants pointed that this species provided significant increment in shoot height, stem diameter, and dry weight of above- and below-ground. According to the authors, this strain could produce GA3 and IAA. Ribeiro and Cardoso (2011), while characterizing the potential of "Plant growth promoting rhizobacteria (PGPR)" bacteria, demonstrated that 18 isolates derived from *Araucaria angustifólia* roots produced IAA. Besides, the increment in growth parameters observed in different studies can be related to the nitrogen fixation capacity and phosphate solubilization, which some bacteria of the genera *Pseudomonas*, *Paenibacillus*, *Klebsiella* and *Enterobacter* have been demonstrated to exhibit, as shown in other studies (Shiomi et al., 2006; Hurek et al., 2002).

Mia et al. (2005) tested plant growth promoting rhizobacterial inoculation along with 33% fertilizer- N in banana and observed significantly increased on bunch yield and fruit physical attributes. The low increment observed in some of the isolates could be attributed to factors such as the banana cultivar, related to the root system or in the composition of the root exudates, and differences in the time and magnitude of the response to the bacterial inoculation (Jaizme-Vega et al., 2004). Even though some authors do not consider the bacterium-host specificity as essential to the promotion of growth (Shishido and Chanway 1998), other researchers emphasize the importance of this specificity for reaching good results (Srinath et al., 2003).

The soil-plant-microorganisms interactions are complex and their individual effects are difficult to be studied. The bacteria observed in this work, in the conditions in which they were isolated, could represent an important component of the growth of ''Prata Anã' banana plantlets. Due to the limited knowledge about the interaction processes between endophytic microorganisms and the environment they colonize, during tests in plants with these microorganisms, the largest possible number of factors associated with the plant (conditions of sanity, nutritional state), the soil (physical, chemical, microbiological factors) and the climate (rain occurrences and irrigation) should be considered. Even though the isolate 07, from the specie *K*. *pneumoniae pneumoniae* has demonstrated great potential as a growth-promoting bacteria in Prata Anã banana plantlets, additional studies must be performed with the aim of evaluating the viability of this isolate in field conditions.

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