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Short communication

Triple combinations with PGPB stimulate plant growth in micropropagated banana plantlets

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ABSTRACT

Twenty different triple combinations of plant growth promoting bacteria (PGPB) were tested on the micropropagated banana plantlet cultivar Prata Anã in terms of growth and nutrient uptake. The experiment was arranged in a randomized design with 22 treatments (20 triple bacteria combinations and two controls) and three replicates. The maximum increments observed for pseudostem height and pseudostem girth were 42.0% and 34.5%, respectively. For aerial dry weight, five triple combinations of PGPB (treatments 6, 8, 9, 10 and 11) presented superior averages compared to control 1 (no bacteria inoculation). The combination of a *Bacillus* sp., repeated twice, plus a *Lysinibacillus* sp. (EB40 + EB53) provided an increase of 174% in the root dry weight. The triple combinations with PGPB also promoted significant differences in the total accumulation in aerial dry weight for all of the analyzed macroelements. Increases in the total accumulation of microelements under triple inoculation were observed for B, Fe and Mn. These results revealed that *B. subtilis* (EB-40), *Bacillus pumilus* (EB-51), *Lysinibacillus* sp. (EB-53), *Paenibacillus* sp. (EB-144) and *Bacillus* sp. (EB-194) have the potential to be exploited as inoculants to improve the plant growth of banana plantlets. Moreover, these selected species can be applied to increase soil microbial diversity, as well as soil quality and soil health.

1. Introduction

In Latin America and Africa, bananas are traditionally propagated using field-obtained suckers, which are often contaminated with soil-borne diseases and pests (Roels et al., 2005). The best strategy to solve these problems is the replacement of the suckers with micropropagated plantlets, which are a source of uniform pest- and disease-free planting material.

Despite the advantages of using micropropagated plantlets, some reports describe that following their transfer to the field, they tend to be less robust and require greater care and attention. Moreover, micropropagated plantlets lack beneficial microorganisms, resulting in an untested defense mechanism (Dubois et al., 2011).

Plant growth promoting bacteria (PGPB) are a group of bacteria with the ability to promote plant growth directly by providing plants with resources or nutrients that they lack by using efficient biological means for nitrogen fixation (Bashan and Levanony, 1990), phosphate solubilization (Rodriguez and Fraga, 1999), iron sequestration (Loper and Buyer, 1991) and modulating growth regulator levels (Patten and Glick, 1996).

The methodology of co-inoculation (double and triple) is not a recent practice in bioinoculation studies; however, this inoculation scheme is also not the most common procedure (Mishra et al., 2011). Elkoca et al. (2010) showed that the triple combination of *Bacillus subtilis*,*Bacillus megaterium* and *Rhizobium* significantly promoted the uptake of macro- and micronutrients for *Phaseolus vulgaris* L.

According to Nowak and Pruski (2002), the re-introduction of endophytic microbes into tissue culture plantlets can occur during *in vitro* co-cultures and during the transplanting phase. Benefits provided by these microorganisms have been reported during the *ex vitro* phase in banana plantlets and even in adult plants, including effects on plant development and yield (Yuan et al., 2013) and improvements in foliar mineral content (Mia et al., 2005; Mia and Shamsuddin, 2010) and disease suppression (Yuan et al., 2013).

Considering the positive effects of PGPB on plant development in banana crops, as indicated by earlier studies, the aim of our research was to investigate the potential use of a triple inoculation

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Table 1
Nutrient content resulting from the chemical analyses of the Bioplant® substrate used during the acclimatization phase in micropropagated banana plantlets cultivar Prata Anã, Janaúba, Minas Gerais, Brazil.

Substrate	pH[1] H_2O	P[2] mg/dm^3	K[2]	Ca[3] cmolc/dm^3	Mg[3]	Al[3]	Al +H[4]	SB	Cu[2] mg/dm^3	Zn[2]	OM[5] 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, Brazil. [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.

with endophytic bacteria in the micropropagated commercial banana cultivar Prata Anã during the acclimatization phase.

2. Materials and methods

Micropropagated banana plantlets of the cultivar Prata Anã (AAB) were provided by the Biotechnology Laboratory of the State University of Montes Claros, Janaúba, Brazil. Plantlets measuring approximately 7 cm in height and with at least three fully developed leaves were transplanted to plastic tubes (50 cm^3) containing the sterilized substrate Bioplant® (Ponte Nova, Brazil) (Table 1). Subsequently, the plantlets were moved to the acclimatization tunnel, with an ambient temperature ranging from 25 to 35 °C and a relative humidity of 90%. After two weeks of acclimatization, the plantlets were transferred into individual 3-L pots containing the same sterilized substrate and maintained in greenhouse conditions for 120 days.

All of the bacterial strains tested in this work were isolated, identified and characterized by Souza et al. (2013) and Andrade et al. (2014), and the nucleotide sequence data reported in this paper have been deposited in the GenBank nucleotide sequence database (Table 2). The final concentrations of bacterial cells in the suspensions were adjusted to 10^8 CFU/mL^{-1}, and 100 mL volumes of the resulting suspensions were used to treat the micropropagated banana plantlets.

Triple combinations containing equal volumes of the three selected strains were inoculated using an irrigation method in which the plantlets and substrate were irrigated with 100 mL of the bacterial suspension at 10^8 CFU mL^{-1} every 15 days (Table 2). The first bacterial suspension application was performed 15 days after transplanting. Control plants (treatments 1 and 2) were irrigated with 100 mL of sterile water.

Hoagland solution (Hoagland and Arnon, 1950), pH 6.8, containing N = 210.1 mg L^{-1}, P = 31.0 mg L^{-1}, K = 234.6 mg L^{-1}, Ca = 200.4 mg L^{-1}, Mg = 48.6 mg L^{-1}, S = 64.2 mg L^{-1}, B = 500 μg L^{-1}, Cu = 20 μg L^{-1}, Cl = 648 μg L^{-1}, Fe = 5022 μg L^{-1}, Mn = 502 μg L^{-1}, Mo = 11 μg L^{-1} and Zn = 50 μg L^{-1}, was applied every 15 days shortly after the inoculum application.

Treatment 1 (control 01) received Hoagland solution[a] and no bacterial inoculation. Treatment 2 (control 02) did not receive Hoagland solution[a] or the bacterial inoculation. Treatments 03–22 received the triple combination with PGPB and modified Hoagland solution[b] (N-free, and the phosphorus was replaced by natural reactive rock phosphate from Algeria with 29% P_2O_5) (Table 2).

The experiment was arranged in a randomized design with 22 treatments and three replicates (Table 2). The evaluations were performed at 120 days after planting. The following characteristics were evaluated: pseudostem height (cm), pseudostem girth (cm), aerial and root fresh weight (g) and aerial and root dry weight (g). To determine the mineral contents, the pseudostems and leaves of the banana plantlets were thoroughly washed and prepared for foliar analysis. The samples were dehydrated in a temperature-controlled fan-ventilated oven at 60 °C for 48 h, then ground to pass through a 1 mm sieve. The Kjeldahl method was used to determine the total N (Tedesco et al., 1985).

Aerial macroelement (P, K, S, Ca and Mg) and microelement (Fe, Cu, Zn, Mn and Na) concentrations were determined using a nitric and perchloric acid method. Phosphorus was measured spectrophotometrically using the indophenol-blue and ascorbic acid method (Braga and Defelipo, 1974). Potassium, calcium, magnesium, iron, manganese, zinc, sodium and copper were determined using a Perkin-Elmer 360 atomic absorption spectrophotometer (Perkin-Elmer, Waltham, Ma, USA) (AOAC, 2005). Boron

Table 2
Bacterial strains and treatments applied as inoculants in micropropagated banana plantlets cultivar Prata Anã during the acclimatization phase, Janaúba, Minas Gerais, Brazil.

Treatments	Description of treatments	Most closely related genus/species
1 (Control 01)	No inoculation and Hoagland solution[a]	–
2 (Control 02)	No inoculation and no Hoagland solution[a]	–
3 (EB04 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus subtilis* + *Lysinibacillus* sp. + *Bacillus* sp.
4 (EB23 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Klebsiella pneumoniae* + *Lysinibacillus* sp. + *Bacillus* sp.
5 (EB25 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus cereus* + *Lysinibacillus* sp. + *Bacillus* sp.
6 (EB40 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp.+ *Bacillus* sp.
7 (EB45 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Lysinibacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
8 (EB47 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp.+ *Bacillus* sp.
9 (EB49 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus licheniformis* + *Lysinibacillus* sp. + *Bacillus* sp.
10 (EB50 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
11 (EB51 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus pumilus* + *Lysinibacillus* sp. + *Bacillus* sp.
12 (EB56 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
13 (EB64 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus pumilus* + *Lysinibacillus* sp. + *Bacillus* sp.
14 (EB87 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus tequilensis* + *Lysinibacillus* sp. + *Bacillus* sp.
15 (EB88 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus flexus* + *Lysinibacillus* sp. + *Bacillus* sp.
16 (EB126 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus subtilis* + *Lysinibacillus* sp. + *Bacillus* sp.
17 (EB127 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Sporolactobacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
18 (EB133 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
19 (EB136 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus subtilis* + *Lysinibacillus* sp. + *Bacillus* sp.
20 (EB144 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Paenibacillus* sp. + *Lysinibacillus* sp. + *Bacillus* sp.
21 (EB169 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus pumilus* + *Lysinibacillus* sp. + *Bacillus* sp.
22 (EB194 + EB53 + EB40)	N + P + IAA + modified Hoagland solution[b]	*Bacillus* sp. + *Lysinibacillus* sp.+ *Bacillus* sp.

The [a] and [b] superscripts are the composition of Hoagland solution described in the materials and methods. N + P + IAA are described as N fixer, P solubilizer and IAA producer.