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The effects of plant growth promoting *Bacillus pumilus* CCIBP-C5 on 'Grande naine' (*Musa* AAA) plants in acclimatization stage

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ABSTRACT

The search of effective and sustainable technological alternatives to replace chemical fertilization it is a challenge for scientists. Positive actions of microorganisms on plants growth are known. Considering these criteria, the objective was to determine the effect of *Bacillus pumilus* CCIBP-C5 on 'Grande naine' (*Musa* AAA) plants in acclimatization stage. The ability to fix atmospheric nitrogen and indoleacetic acid production *in vitro* was determined. Furthermore, micropropagated *Musa* sp. plants were inoculated by immersion of roots in bacterial suspension. *B. pumilus* was able to grow in culture media without nitrogen, and produced 28.9 µg ml^{-1} of indoleacetic acid. Besides, *B. pumilus* increased significantly the height and stem thickness, modified roots architecture and enhanced fresh and dry weight of plants. The bacteria strain CCIBP-C5 favorably influenced the growth of plants.

Key words: Indoleacetic acid, PGPB, growth promotion

Efecto de bacteria promotora del crecimiento vegetal *Bacillus pumilus* CCIBP-C5 sobre plantas de 'Grande naine' (*Musa* AAA) en fase de aclimatización

RESUMEN

La búsqueda de alternativas tecnológicas eficaces y sostenibles para la sustitución de la fertilización química es un reto para los científicos. Se conoce de efectos positivos de microorganismos sobre el crecimiento vegetal. Teniendo en cuenta estos criterios, el objetivo fue determinar el efecto de *Bacillus pumilus* CCIBP-C5 sobre plantas de 'Grande naine' (*Musa* AAA) en fase de aclimatización. Se determinó *in vitro* la capacidad de fijar el nitrógeno atmosférico y de producir ácido indol acético. Además, plantas de *Musa* sp. micropropagadas se inocularon por inmersión de las raíces en la suspensión bacteriana. *B. pumilus* fue capaz de crecer en medio de cultivo sin nitrógeno y produjo 28.9 µg ml^{-1} de ácido indol acético. Además, la cepa *B. pumilus* aumentó significativamente la altura, el grosor del tallo, modificó la arquitectura de las raíces y aumentó la masa fresca y seca de las plantas. La cepa CCIBP-C5 influenció favorablemente el crecimiento de las plantas.

Palabras clave: ácido indol acético, PGPB, promoción de crecimiento

INTRODUCTION

One of the critical points in the bananas and plantains production is related to fertilization. Generally, require large amounts of nitrogen ($N_{2)}$ and potassium (K) followed by phosphorus (P), calcium (Ca) and magnesium (Mg) to maintain high yields (Robinson, 1996).

However, the increased employment of chemical fertilizers is undesirable, because (1) production is an energetically expensive process and most of the energy is provided by the consumption of nonrenewable sources such as fossil fuels and (2) a substantial pollution is caused by the production and use of mineral nitrogen fertilizers (Mia *et al.,* 2010). Consequently, search effective and sustainable technological alternatives to replace chemical fertilization it is a challenge for scientists.

The possibility to use microorganisms that live associated with plants is a promising alternative for a sustainable agriculture. Many researchers suggested that Plant Growth Promoting Bacteria (PGPB) are effective as a bioenhancer and biofertilizer for banana plants. For instant, Mia *et al*. (2005) found enhanced root formation in bananas, when PGPB strain was inoculated in plants. Meanwhile, the effect of the combined inoculation of the arbuscular mycorrhiza *Glomus manihotis* and a rhizobacteria consortium of *Bacillus* spp. on micropropagated banana plantlets, during the acclimatization stage increased growth parameters like shoot length, leaf area, total fresh and aerial dry weight (Rodríguez-Romero *et al*., 2005). Also, PGPB inoculation in bananas showed a significant amount of nitrogen fixation (Mia *et al*., 2010).

Current agricultural use bacterial-based products as pesticides, fungicides and stimulators of plant growth (Pérez-García *et al*., 2011). Considering these criteria, the objective was to determine the effect of *Bacillus pumilus* CCIBP-C5 on 'Grande naine' (*Musa* AAA) plants in acclimatization stage.

MATERIALS AND METHODS

Plant material

In vitro plants of 'Grande naine' (*Musa* AAA) were propagated by organogenesis according to the protocol described by Orellana (2004). After them, plants were placed in polythene bags with sterilized mixture of compost and zeolite (80:20). The plants were arranged in a greenhouse, according to a completely randomized design.

Bacterial strains and culture conditions

Bacillus pumilus CCIBP-C5 belonging to the Microbial Culture Collection of Applied Microbiology Laboratory from Instituto de Biotecnología de Las Plantas was used. *B. pumilus* was isolated from banana phyllosphere. Beside, this strain have *in vitro* antifungal activity against *Mycosphaerella fijiensis* (Poveda *et al.,* 2010).

Bacterial strain was grown in 250 ml flask containing 100 ml of Nutrient Broth (Fluka) medium at 30°C and 120 rpm shaking until exponential growth phase, OD_{600} = 0.1, equivalent to 10^9 colony-forming units (cfu) ml^{-1}.

An aliquot of biological material was taken from pure culture to evaluate the nitrogen fixation, Indoleacetic acid (IAA) quantification and plant promotion assay.

Bacterial strains characterization

In order to determine the ability to fix atmospheric nitrogen, *B. pumilus* strain was streaked onto nitrogen-free Winogradsky medium (Krieg and Holt, 1984). Petri dishes was incubated at 28°C for 72 h. It was reported as positive result if the strain was able to grow in this culture medium. Nutrient Agar culture medium (Fluka) was used as a growth control.

IAA production was determined according to colorimetric method (Patten and Glick, 2002) as described below. The *B. pumilus* strain was grown in culture medium Tryptone Soya Broth (BioCen) at 28°C for 48 h in darkness. Cells were removed by centrifugation (10 000 g x 15 min) and 1.0 ml of the supernatant was added to 500 µl Salkowsky reagent. The mixture was incubated at 30°C and darkness for 30 min. Absorbance was measured at 530 nm (UV-visible spectrophotometer, Genesys 6, Thermo Electron Corporation, USA). The result was reported in µg ml^{-1} calculated from an IAA (Duchefa) standard curve (1.25, 2.50, 5.00, 10.00, 20.00, 40.00 µg ml^{-1}).

Plant growth promotion

The plants were inoculated with bacterial suspension, by dipping roots for 20 min. Plants without bacteria treatment were used as control. The evaluation of the plants were performed weekly until 60 days after inoculation. The plant height (cm), stem thickness (cm), root length (cm), number of leaves and fresh and dry weight (g) of foliage and roots were determined. The dry weight was determined by using an oven at 70°C for 72 h. In each treatment, ten plants were used and the experiment was repeated twice.

Statistical analysis

As data did not meet the assumptions of normality and homogeneity of variances, it were submitted to non-parametric test ($p \leq 0.05$) using a Statistic Package for Social Science (SPSS) v.20 for Windows.

RESULTS AND DISCUSSION

B. pumilus CCIBP-C5 was able to grow in culture medium without nitrogen. Also, *B. pumilus* produced 28.9 µg ml^{-1} of indoleacetic acid *in vitro* condition.

The selection of bacterial strains with the ability of nitrogen fixation and IAA production, as CCIBP-C5, will contribute to develop of new biofertilizer products. Results of different research have been indicated that biological nitrogen fixation process accounts for 65% of the nitrogen utilized in agriculture, and to be important in future sustainable crop production systems (Matiru and Dakora, 2004). Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth and production of food and feed (Hayat *et al*., 2010). The employ of N_2 fixing and phosphate solubilized bacteria in agriculture could to reduce the chemical fertilization use (Mia *et al*. 2005).

Some authors have been previously indicated the favourable effects of IAA on plant growth. This hormone increases the rate of xylem and root development (lateral and adventitious), control processes of vegetative growth and affects photosynthesis (Tsavkelova *et al.*, 2006). Besides, IAA synthesized by bacteria may be involved at different levels in plant-bacterial interactions. In particular, plant growth promotion and root nodulation (Glick, 2012).

Plant growth promotion after the inoculation of *B. pumilus* CCIBP-C5 was observed. At 60 days after inoculation, CCIBP-C5 increased plant height (Figure 1) and stem thickness (Figure 2) respect to control.

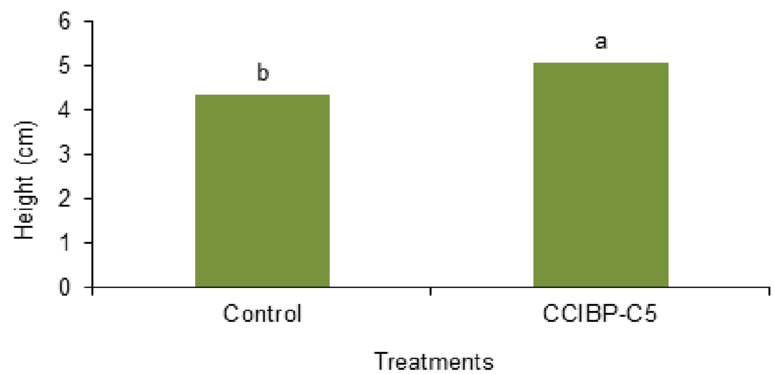

Figure 1. Height of 'Grande naine' (*Musa* AAA) plants after 60 days of inoculation with *B. pumilus* CCIBP-C5 in acclimatization stage. Different letters above bar indicate significant differences between treatments by Mann-Whitney test for $p \leq 0.05$.

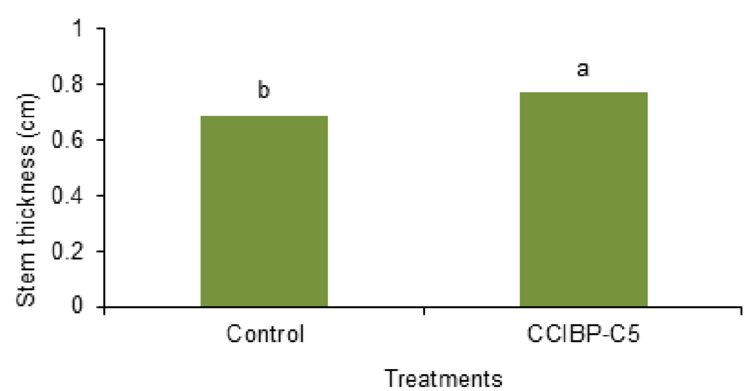

Figure 2. Stem thickness of 'Grande naine' (*Musa* AAA) plants after 60 days of inoculation with *B. pumilus* CCIBP-C5 in acclimatization stage. Different letters above bar indicate significant differences between treatments by Mann-Whitney test for $p \leq 0.05$.

An increase in plant height has been observed by other authors like Galindo-Menéndez *et al*. (2009) using PGPB. Theirs found that a native strain of *Azotobacter* sp. Tu-24 stimulated the growth of *Musa* sp. cv. 'FHIA-18' plants similar to treatment with BIOBRAS (commercial stimulators) and superior to control with water.

On the other hand, plants inoculated with *B. pumilus* CCIBP-C5 not showed differences in number of leaves per plant or root length respect to control with water. However, plants treated with the bacterial strain shows different radicle morphology, presence of secondary lateral roots (Figure 3) and significantly increase of the fresh and dry weight (Figure 4).

Figure 3. Banana plants cv. 'Grande Naine' (*Musa* AAA) after 60 days in acclimatization stage . A) Control, B) Inoculated with *B. pumilus* CCIBP-C5.

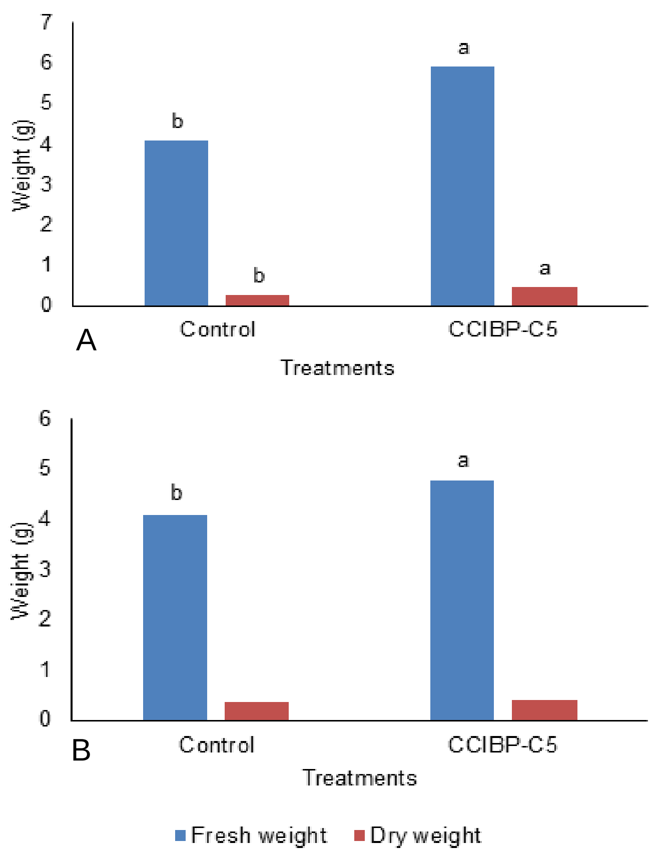

Figure 4. Fresh and dry weight of 'Grande naine' (*Musa* AAA) plants after 60 days of inoculation with *B. pumilus* CCIBP-C5 in acclimatization stage. A) Foliage, B) Roots. Different letters above bar indicate significant differences between treatments by Mann-Whitney test for p$\leq$0.05.

The result is in accordance with *in vitro* IAA production by CCIBP-C5 strain and previous reports. Is known that essential plant nutrients are taken up from the soil by roots. In this sense, good root growth is characteristic of auxin effect produced by bacteria and is one of the parameters used to determine the effectiveness of certain bacteria (Torres-Rubio *et al.,* 2000). Many PGPR stimulates the root growth, sometimes via production of phytohormones by the plant or by the bacteria (Lucy *et al*., 2004). Promotion of root growth is considered a marker by which the beneficial effect of PGPB is measured. Overall, bacterial IAA increases root surface area and length, and thereby provides greater access to soil nutrients for plants (Glick, 2012).

According to the results of Rodríguez-Romero *et al*. (2005), banana plants treated with *Bacillus* sp. alone and in combination with *Glomus manihotis* had significantly increase in total fresh and aerial dry weight respect to control. A plant can grow vigorously if it contains much amount of fresh and dry weight (Mathivanan *et al*., 2014).

Bacteria have the potential to contribute in the development of sustainable agricultural systems (Mia *et al.,* 2010). This contribution is by three different ways: synthesizing particular compounds such us auxin, facilitating the uptake of certain nutrients from the soil and lessening or preventing the plants diseases (Glick, 2012). Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective biofertilizer.

In the case of *Bacillus* strains several reports indicated applications to enhance the growth of agricultural crops, wild plants, trees, microalgae, and model plants, through different mechanisms of plant growth-promotion (Kloepper *et al*., 2004; Bashan *et al*., 2010). In this work, *B. pumilus* CCIBP-C5 strain shows good promise as an inoculant for banana plant growth promotion in acclimatization stage. According to these results, the use of phyllosphere bacterial strains in sustainable agriculture could be an option to reduce the amounts of external inputs and time required for *Musa* spp. acclimatization.

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