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Evaluation of phosphodissolvent IAA producing strains of *Trichoderma*, spp. through biometric response of *Phaseolus vulgaris* L

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Abstract

Studies report that in addition to biocontrol effects, *Trichoderma* spp., exhibit phosphodissolvent and indole acetic acid (IAA) production capacities. In greenhouse laboratory conditions, the effects of four native strains of *Trichoderma* spp., were evaluated on the germination and growth of beans. Inoculants of each strain were prepared in concentrations of 10^4, 10^6, and 10^8 spores mL^{-1}, which were then used to inoculate bean seeds and seedlings. The biometric variables evaluated were number of germinated seeds, germination percentage, germination velocity index, and median germination time. Later, germinated seeds were taken to greenhouse conditions and over the course of 90 days, height of seedlings, circumference of stalks, number of leaves, length of roots, number and mass of fruits, and overall dry mass were measured. The TRIC13 strain, with concentrations of 7 x 10^8 spores mL^{-1} , showed better results than the rest of the strains evaluated with respect to seed germination. However, in relation to growth and germination of bean plants, concentrations of 7 x 10^4 spores mL^{-1} were more efficient in all treatments evaluated. Considering the biometric variables evaluated, the study showed an ability of strains of *Trichoderma* to promote the growth and efficacy of greenhouse bean plants.

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Introduction

Trichoderma spp., have been reported to be fungal promoters of vegetal growth (PGPF: Plant Growth Promoting Fungi) (Yedidia *et. al.* 1999; Harman 2000; Gravel *et al.,* 2007; Vinale *et al.,* 2008; Achá 2008), an action that was studied through the production of metabolites such as (i) specific amino acid inductors of IAA hormones, such as L-tryptophan (L-Trp) (Gravel *et al.,* 2007), (ii) pathogenic biocontrol effects (Vinale *et al.,* 2006; Vinale *et al.,* 2008), and (iii) the direct inoculation of *Trichoderma* spp., has achieved an increase in the velocity and percentage of seed germination, as well as reducing the effects caused by environmental stress conditions (Bjorkman *et al.,* 1998). These characteristics confirm that it may be possible to use *Trichoderma* in the biotechnology industry.

Under natural soil conditions, it is possible that various species of the genera may synthesize a higher quantity of IAA as compared to pure cultures in laboratory conditions. This is possibly due to synergistic effects with other microorganisms and interactions with organic material in the soil. It has been established that the synthesis of auxins and related substances occurs in larger proportions in the rhizosphere due to the fact that it is favored by root exudates and substances derived from the lysis of vegetal cells (Hinojosa *et al.,* 2008; Katsnelson and Sirois 1961 in: Arshad and Frankenberger 1993).

In addition, the presence of IAA of microbial origin has been demonstrated in the interior of plants (Libbert *et al.,* 1969 in: Arshad and Frankenberger 1993). Thus, soil microorganismal IAA producers may be favorable to vegetal growth, as they make these substances available for plants, initiating hormonal effects that lead principally to the promotion and synthesis of proteins and cellular division and lengthening, which produce macroscopic responses such as germination and development; the proportional growth of the plant, or the separate growth of different organs in certain phenological stages; and modification of apical dominance and plant architecture. Two key effects for plant nutrition are the levels of rhizogenesis and root lengthening, which translate into better anchoring and capacity to explore larger quantities of soil, favoring the uptake of nutrients.

In consonance with the above, various investigations have reported that the association of phosphate solubilizing microorganisms (PSM) with the rhizospheres of plants contributes to their supplying phosphorous in an ecosystem in which it is a limiting resource.

These IAA producing PSM microorganisms offer an important microbial germplasm bank that may be utilized in agricultural activities (Zúñiga and Becerra 2014; Osorno and Osorio 2014; Trolove *et al.,* 2003; Gyaneshwar *et al.,* 2002; Richardson 2001).

Fungi exhibit various mechanisms for solubilizing inorganic P compounds, among those that stand out are the production of lithic enzymes during the decomposition of organic matter (Marschner 2008; Bar-Yosef *et al.,* 1999; Iyamuremye *et al.,* 1996), and organic acids that generate competition between organic anions produced and phosphate ions in absorption sites on the surfaces of clayey soil minerals (Bolan *et al.,* 1994).

Some of the organic acids commonly associated with microbial P solubilization by fungi are: gluconic (Rodríguez *et al.,* 2006; Bar-Yosef *et al.,* 1999), oxalic, citric (Osorio 2008; Kim *et al.,* 1997; Kucey and Leggett 1989), lactic, tartaric, and aspartic (Venkateswardu *et al.,* 1984).

These acids are products of microbial metabolism, in some cases, due to oxidative respiration or through fermentation of carbonaceous substrates (ex: glucose) (Prescott *et al.,* 2004; Mathews *et al.,* 2002; Atlas and Barta 1998). Other mechanisms proposed are the excretion of protons due to the assimilation of NH_4^+ by microorganisms (Whitelaw 2000; Abd-Alla 1994; Roos and Luckner 1984; Kucey 1983); desorption of P ions at absorption sites (He and Zhu 1998 1997); and chelation of Al^{3+} and Fe^{3+} (Marschner 2008; Bar-

Yosef *et al.,* 1999; Iyamuremye *et al.,* 1996).

However, even though there are wide-ranging reports of the beneficial effects of PSM's, frequently, the results are inconsistent (Chen *et al.,* 2007a; Mc Spadden Gardener, 2004; Reva *et al.,* 2004; Garbeva *et al.,* 2003). It is presumed that this inconsistency is due to the fact that a single inoculation of microorganisms in the soil-plant system alters the dynamics of the rhizospheric ecosystem (de Freitas *et al.,* 1997; Habte and Osorio 2001; Chigineva *et al.,* 2011). It is also known that this differential response depends on the vegetal species (Flach*et al.,* 1987; Gregory 2006), the mineralogical composition of the soil (Boul and Eswaran 2000), and the interactions between different microbial functional groups of the soil (Chigineva *et al.,* 2011; Vessey 2003; Habte and Osorio 2001).

Reports done by Caipo *et al.,* (2002), also show that the type and quantity of inoculant affects the cellular physiology of microorganisms and the host plant; as well as the availability of carbon substrate(s) as an energy source (Caldeira *et al.,* 2008; Knox *et al.,* 2000), the competitiveness with other autochthonous soil microorganisms (Collados 2006; Silvieira *et al.,* 2003), and environmental conditions (Johnson *et al.,* 1997) are indicated in the differential response of crops to microbial inoculation.

In the search for microorganisms that synthesize IAA and solubilize phosphates, studies have reported that *Trichoderma*, in addition to having biocontrol effects on pathogens, confers other benefits on plants through the decomposition of organic matter and the liberation of nutrients in available forms for the plant (Howell 2003; Godes 2007) and shows phosphate solubilizing activities (Valencia *et al.,* 2007; Valero 2007; Vera *et al.,* 2002), which is why it is frequently utilized as a biofertilizing organism in different commercial products (Moreno *et al.,* 2007). *Trichoderma* promotes the growth and development of crops by producing metabolites that stimulate the processes of vegetal growth and development (Hoyo-Carvajal *et al.,* 2009; Sutton and Peng 1993) due to its degradation of seed episperm and its intervention in respiratory processes during germination.

It is also known to accelerate the development of primary meristematic tissues, which allows for an increase in plant volume, height, and mass (Moity 1982; Miranda *et al.,* 1998; Gravel *et al,* 2007; Shoresh and Harman 2008a; Shoresh and Harman 2008b). Another inherent characteristic of *Trichoderma* is that it has the capacity to multiply in the soil and colonize plant roots, thus liberating growth factors (auxins, gibberellins, and cytokinins), which stimulate plant germination and development (Altomare *et al.,* 1999).

The production of 3-indole acetic (IAA) acid has been reported for *T. harzianum*. This substance acts as a vegetal hormone favoring the development of the root system (Valencia *et al.,* 2005), due to the fact that it is a catalyst or accelerator of primary meristematic tissue in young parts of the plant, accelerating cellular reproduction; thus allowing treated plants to develop much faster than plants that have not been treated with this microorganism (Valencia *et al.,* 2007). *T. harzianum* has also been reported to be a promoter of vegetal growth in eggplant, vetch, beans, coffee, tomato, potato, and forest species (Zambrano 1989; Börkman *et al.,* 1998; Dandurand and Knudsen 1993), among other benefits.

Due to the relevance of this fungus, the hypothesis that the biometric response of plants is a function of the strain and inoculant concentration evaluated was proposed; which will contribute to the development of strategies for the integrated management of crops due to the elaboration, evaluation, and application of bioproducts with multifunctional effects caused by its biocontrolling activities on pathogens, in addition to its phytostimulating and biofertilizing effects.

The proposed objective was the evaluation of the biopromoter effects of different strains of *Trichoderma* spp. with phosphate solubilizing and 3-indole acetic acid (IAA) production capacities at different inoculant concentrations on the germination

and development of beans (*Phaseolus vulgaris*).

Materials and methods

This investigation was realized in the Biotechnology Laboratories and the coverage zone of the Faculty of Sciences and Health of the Mayor College of Antioquia University Institution and in the Ecology and Environmental Conservation and Vegetal Health Laboratories of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Medellín campus (6°15´ N, 75°34´ W, 1450 m of altitude).

Experiment 1: In vitro germination of seeds

The capacities of strains of *Trichoderma* spp., to dissolve phosphatic rock (PR) and to produce IAA efficiently were tested. To do this, circles of filter paper were placed over cotton in germination cells and sterilized in an autoclave at 120°C and 1.2 kg cm^{-2} for 20 minutes. Suspensions of spores were prepared with 1 mL $CaCl_2 \cdot 2H_2O$ 0.01*M* at a concentration of $7x10^4$, $7x10^6$ y $7x10^8$ spores per mL for each morphotype of Trichoderma with PR solubilization and IAA production capacities (Cubillos-Hinojosa *et al.,* 2009; Capuchino and Sherman 1998). Each experimental unit (germination cell) was inoculated with 1 mL of suspension of the concentration of each corresponding strain. Later, sterile distilled water was added to each cell until its support was saturated. As a control, the uninoculated cells received 1 mL of a solution of $CaCl_2 \cdot 2H_2O$ 0.01*M*. Next, 24 bean seeds that were previously disinfected with hypochlorite at 1% ethanol and 70% were placed in each cell.

The cells were incubated in complete darkness at 27 ± 1°C, for 15 días. Each treatment had 4 replicas. Daily observations were done for 15 days, during which the number of germinated seeds was registered for those in which the roots reached 2 mm in length. From the data collected, the percentage of germination (PG), expressed as the total percentage of germinated seeds at 15 days was determined. The index of velocity of germination (IVG) was calculated according to the formula proposed by Brown and Mayer (1988). The median germination time (MGT) was calculated according to the methodology proposed by García *et al.,* (1982) and Cubillos-Hinojosa *et al.,* (2009).

Experiment 2. Evaluation of bean plants at the greenhouse level

Vegetal material

The model plant used in this study was the *Cargamanto rojo (Phaseolus vulgaris* L) bean variety. Seeds were previously selected in the field by characteristics, with plants being selected for good growth, development, and health. Later, the best vines were chosen from the middle third of the plants. From these plants, the best seeds were chosen by size and shape. Before planting, the seeds were disinfected with a solution of sodium hypochlorite at 1 % (v/v) and ethanol at 70% (v/v) for 30 seconds, respectively, and then washed twice with sterile distilled water in two repetitions.

The seeds were planted in 5 kg bags which contained a sterile mixture of organic material, sand, and vermiculite (3:1:0.5). The bags were then placed in the coverage zone of the Mayor College of Antioquia University Institution, Colombia.

Preparation of fungal inoculates

The strains of *Trichoderma* were isolated and selected from rhizospheric soil and bean root segments from cultivations of beans at 30, 60, and 90 days after planting, established in the Agrarial Station San Pablo, on the property of the Universidad Nacional de Colombia, located in the municipality of Rionegro (Antioquia).

Four strains of *Trichoderma* were utilized for their capacities to solubilize P and produce IAA; which were referenced as TRIC7, TRIC11, TRIC13, and TRIC48; donated by the Biotechnology Laboratory of the Mayor College of Antioquia University Institution.

These were cultivated in antibiotized PDA media with 300 mg L^{-1} Streptomycin Sulfate and 100 mg L^{-1} chloramphenicol (SIGMA) in complete darkness. All petri dishes were incubated for 24 hours at 25±1°C. A total of 20 petri dishes were planted. They were then preserved at 4°C until their evaluation.

After 8 days of growth, a 5 mm diameter circle was cut in the centers of the colonies. Each disk was then put into a 1.5 mL microvial with 1 mL of sterile distilled water and Tween 80 and agitated in a vortex for 1 min. From these vials, 1 mL of solution was taken, which was then added to test tubes that contained 9 mL of sterile 0.85% NaCl solution. Eight (8) successive decimal dilutions were then done for each suspension from 10^{-1} to 10^{-8}, from which, four (4) spore dilution counts were done using a Neubauer chamber.

The viability of the spores to be inoculated in the PR solubilization broths was realized with fluorescein acetate following the procedure reported by Calich *et al.,* (1979). Simultaneously, in order to compare counting methods (Neubauer chamber and UFC count in Petri dishes), 1 mL of each dilution was planted in Standard Plate Count (SPC) (SIGMA) four-petri dish containers for a total of 36 petri dishes planted for each fungal strain.

The petri dishes were incubated at 25±1°C for 12 hours. Later, a count of germinated spores was done by dilution. Each axenic culture was obtained using the monosporic methodology reported by Zúñiga *et al.,* (2010), with the goal of evaluating cytogenetically homogenous strains. These strains were stored at 4°C until their evaluation. Spore suspensions were prepared for each fungal strain with PR solubilizing and IAA producing capacities using 1mL $CaCl_2 \cdot 2H_2O$ 0.01*M* at a concentration of $7x10^4$, $7x10^6$, and $7x10^8$ spores per mL for their later inoculation at the greenhouse level.

Preparation of soil and inoculation of *Trichoderma* spp. at the greenhouse level.

The soil evaluated corresponded to the A horizon (0 to 20 cm) of an entisol from the San Pablo Agrarial Station, on the property of the Universidad Nacional de Colombia, in the municipality of Rionegro (Antioquia). It was previously macerated and ground to 4mm. It was then sterilized at 120°C and 1.2 kg cm^{-2} for 20 minutos for two continuous autoclave cycles. Next, it was transfered to plots that contained five (5) kg of soil. Based on the results of physiochemical analyses, the entisols were fertilized with 0.25 g $(NH_4)_2SO_4$ per kg of soil. At the moment of planting, in the corresponding treatments, 100g of soil (dry base) was uniformly mixed with 25 mL of fungal inoculant following the treatment. At each plot, 2.5 g • kg^{-1} of unacidulated phosphoric rock (PR) was added. As controls, the uninoculated plots received 25 mL of $CaCl_2 \cdot 2H_2O$ 0.01*M* solution.

Daily, the plants were watered with tapwater. The plants grew for 90 days, after which all the treatments were taken apart. In each experimental unit, the following biometric parameters were evaluated: a) leaf quantity (number): count of cotyledonous leaves that were truly photosynthetically active in each plant; b) quantity of fruit (number): count of mature vines with seeds in each replica of the treatments; c) total length of the plant (cm): measured from the apex of the root to the most apical leaf of each plant sampled; d) length of sprout (cm): measured from the longest apical leaf to the neck of the root; e) root length (cm): measured from the apex of the principal root to the root neck of each plant sampled; f) dry mass (g): samples were dried in a stove at 60°C until they reached a constant dry weight.

Experimental design and statistical analysis

The experimental design was completely random. For experiment 1, the treatments had a 4x3 (Four (4) strains of *Trichoderma* spp. and three (3) doses of evaluated inoculant) factorial arrangement and one (1) uninoculated control. The dependent variables were the percentage of seed germination (PG), the index of velocity of germination (IVG), and the median time of germination (MTG). Each treatment had 4 replicas.

For experiment 2, the treatments had a 4x3 (Four (4) strains of *Trichoderma* spp., and three (3) doses of evaluated inoculant) factorial arrangement and one (1) uninoculated control.

The dependent variables were plant biomass and P

foliar concentration (mg L^{-1}). Each treatment had 4 replicas. The experimental units were distributed in a random manner.

The results were analyzed through the use of an analysis of variance (ANOVA), through which, assumptions and residuals were determined. A Duncan test was utilized for the separation of media. The analyses were done with a (P) ≤ 0.05 level of significance. All analyses were completed with the Statgraphic statistical software, version Centurion XVI.

Results

Experiment 1. In vitro germination of seeds

The data obtained for the daily accumulated germination percentage show that all treatments except TRIC48 10^4 (43.4%) increased the germination of bean seeds. The treatments of the TRIC7, TRIC11, and TRIC13 strains showed PG values that oscillated between 60.7% and 93.1%. The PG value that TRIC48 10^4 showed was comparable with the control treatment, which had a germination percentage of 53.8% (Table 1).

Table 1. Germination percentage (PG), Index of velocity of germination (IVG), and Median time of germination (MTG) of bean seeds inoculated with different strains of *Trichoderma* spp., at the *in vitro* level.

Tratamiento	PG	IVG	TMG
TRIC7-10^4	44.1abc	1.325abc	7.2ab
TRIC7-10^6	64.4abc	1.725abc	6.7ab
TRIC7-10^8	93.1a	1.962abc	5.8a
TRIC11-10^4	53.8c	1.294c	9.14b
TRIC11-10^6	65.7abc	1.828abc	6.5ab
TRIC11-10^8	91.1ab	2.275ab	6.9ab
TRIC13-10^4	60.7abc	1.221abc	7.4ab
TRIC13-10^6	66.7abc	1.821abc	6.6ab
TRIC13-10^8	91.7ab	2.621a	5.9a
TRIC48-10^4	43.3c	0.849bc	7.17ab
TRIC48-10^6	53.0c	0.990c	9.09b
TRIC48-10^8	52.3c	0.999c	9.00b
Testigo	53.4c	0.994c	9.13b

The averages with distinct letters indicate significant differences according to the Duncan test (P≤0.05).

The TRIC7 10^8, TRIC11 10^8, and TRIC 13^8 treatments showed the highest germination percentages (PG), with values of 93.1%, 91.1%, and 91.7%, respectively. There was a significant statistical difference (P≤0.05) with respect to the control treatment. The results generated show a biopromoter effect with growth function due to the higher concentrations of PG inoculant.

The index of velocity of germination (IVG) for the treatments of the TRIC7, TRIC11, and TRIC13 strains were larger than those of the treatments of the TRIC48 strain and the control treatment (Table 1). The results above indicate that the velocity of germination of bean seeds inoculated with the TRIC7, TRIC11, and TRIC13 strains have larger IVG values that oscillate between 1.221 and 2.621 with respect to the control, the IVG of which was 0.994, showing a significant difference (P≤0.05). These higher IVG values confirm a relationship that is directly proportional between concentrations of inoculate and IVG, due to the fact that increasing the concentration of inoculant increased the velocity of seed germination.

In relation to the median time of germination of seeds (MTG), which indicates the time required for 50% of the seeds to germinate, the results obtained show higher MTG values for the control treatment, TRIC11 10^4, TRIC48 10^6, and TRIC48 10^8 with MTG values of 9.13, 9.14, 9.09, and 9.00, respectively, without significant differences between the treatments

mentioned. The TRIC7 10^8 and TRIC13 10^8 treatments showed significant differences ($P \leq 0.05$) in relation to all the treatments evaluated, including the control (Table 1), with MTG values of 5.8 and 5.9, respectively. With these results, it can be deduced that the type of strain and the concentration of inoculant have the same influence on MTG as bean seeds, (e.g. decreasing MTG).

The results reported in this investigation show that the TRIC7, TRIC11, and TRIC13 strains have more optimal PG, IVG and MTG values than the TRC48 strain and control treatment at concentrations of 10^6 and 10^8 spores per mL. This fact may be attributed to the better adaptation, colonization, and degradation of episperm of the bean seeds.

Table 2. Treatments evaluated in beans by *Trichoderma* spp. strain and inoculant concentration.

Strain	Treatment	Inoculum concentration as treatment (spores mL)
TRIC7	T1	7×10^4
	T2	7×10^6
	T3	7×10^8
TRIC11	T4	7×10^4
	T5	7×10^6
	T6	7×10^8
TRIC13	T7	7×10^4
	T8	7×10^6
	T9	7×10^8
TRIC48	T10	7×10^4
	T11	7×10^6
	T12	7×10^8
Control	T13	Uninoculated

Experiment 2. Evaluation of bean plants at the greenhouse level.

The treatments evaluated and the results of the two biometric analyses can be seen in Tables 2 and 3. These data sets correspond to the average values of the bioassays, each one with four replicas for the different treatments:

Quantity of leaves (number)

The sprouting of cotyledonous leaves was seen simultaneously in all treatments on the tenth (10^{th}) day after germination. Higher growth, development, and production of true leaves per plant was seen in the TRIC13 10^4, TRIC13 10^6, TRIC13 10^8, TRIC7 10^4 , and TRIC7 10^6 treatments, respectively (Table 3). The total number of healthy leaves in these treatments were: 29.0, 22.75, 20.75, 24.27, and 22.00; respectively. A significant interaction was observed between strain and inoculant concentration evaluated ($P \leq 0.01$) in the variable Number of Healthy Leaves (NHS).

Quantity of fruits (number)

Counting of mature vines with seeds was done at the time of the breakdown of the experiment (90 days after planting).

The highest number of vines were obtained in the TRIC13 10^4, TRIC13 10^6, TRIC7 10^4 , and TRIC7 10^6 treatments, with values of 12.0, 10.0, 9.0, and 8.5; respectively, data that are highly significant ($P \leq 0.01$) with respect to the rest of the treatments (Table 3).

Total plant length (cm)

Plants that were evaluated in the TRIC13 10^4 and TRIC13 10^6 treatments were longer on average, with highly significant differences ($P \leq 0.01$) with respect to the rest of the treatments. In these treatments, the plants reached values of 128.27 cm and 122.50 cm, respectively (Table 3). A highly significant strain effect ($P \leq 0.01$) was observed with respect to the concentration of inoculate in all the other treatments (Table 3).

Table 3. Average of biometric parameters of *Phaseolus vulgaris* L. plants inoculated with different strains of *Trichoderma* spp. and inoculant concentrations.

Treatment	TPL (cm)	TPDM* (g)	SL (cm)	SDM (g)	RL (cm)	RDM (g)	LN		NFr	FrDM(g)
							D	H		
TRIC7 10^4	114,50	26,60a	62,25	12,73	52,25	0,50	0,50	24,25	9,0	13,38
TRIC7 10^6	108,75	24,88a	61,75	12,75	47,00	0,48	3,25	22,00	8,5	11,65
TRIC7 10^8	107,25	18,93b	61,75	9,40	45,50	0,40	4,50	19,75	5,0	9,53
TRIC11 10^4	117,00	12,53cd	69,00	5,73	48,00	0,43	2,00	18,75	7,5	6,38
TRIC11 10^6	116,50	11,88d	66,50	6,73	50,00	0,48	2,50	18,75	5,5	4,68
TRIC11 10^8	111,25	10,13e	65,00	4,95	46,25	0,40	4,50	17,25	3,5	4,78
TRIC13 10^4	128,25	29,68a	83,50	14,20	44,75	0,35	2,75	29,00	12,0	15,13
TRIC13 10^6	122,50	27,08a	67,25	12,95	54,75	0,48	2,75	22,75	10,0	13,65
TRIC13 10^8	115,75	16,55bc	60,75	8,20	55,00	0,48	4,50	20,00	7,5	7,88
TRIC48 10^4	109,25	7,80de	60,50	3,00	48,75	0,43	3,75	17,25	4,25	4,38
TRIC48 10^6	103,75	7,65e	61,75	2,43	42,00	0,30	7,75	16,50	4,75	4,93
TRIC48 10^8	100,1	7,97de	60,3	2,30	43,0	0,40	7,78	17,3	4,50	4,38
Testigo	99,50	7,75e	59,00	4,95	40,50	0,38	7,75	16,25	5,50	2,43

TPL= Total plant length; TPDM= Total plant dry mass; SL= Sprout length; SDM= Sprout dry mass; RL= Root length; RDM= Root dry mass; LN= Leaf number; H= Healthy leaves; D= Damaged leaves; NFr= Number of fruits; FrDM= Fruit dry mass. *Averages with distinct letters indicate significant differences according to the Duncan test ($P \leq 0.05$).

Total dry mass of the plant (g)

The TRIC13 10^4 (29.68 g), TRIC13 10^6 (27.08 g), TRIC7 10^4 (26.60 g), andTRIC7 10^6 (24.88 g) treatments had higher average dry masses than the plants with significant differences ($P \leq 0.05$) between the TRIC13 10^4 and TRIC7 10^6 treatments (Table 3). However, upon separately measuring the dry mass of the sprouts of TRIC13 10^4 (14.20 g), it showed better behavior than TRIC13 10^6 (12.95 g), TRIC7 10^4 (12.73 g), and TRIC7 10^6 (12.75 g) with significant differences ($P \leq 0.05$) between TRIC13 10^4 and the other treatments mentioned. The same behavior was obtained with the average dry mass of fruits in comparison to other treatments evaluated, the values of which were inferior (Table 3). In comparing media, the treatments that had 7 x 10^4 esporas mL^{-1} showed an average effect that was higher in the response variables LPT, PSPT, LV, PSV, LR, PSR, NH: S and D, Nfr, and PSFr, with significant differences shown at concentrations of 7 x 10^6 and 7 x 10^8 spores mL^{-1} and with the control, in which the values of the variables described decreased. Finally, the treatments inoculated with the TRIC48 strain at any concentration of inoculate evaluated showed similar behavior to the control, except in the case of the variable PSFr, the value of which was higher than the TRIC48 strain at all inoculate concentrations evaluated with respect to the control treatment, and with significant differences ($P \leq 0.05$) between treatments; this P value suggests a higher effect on the accumulation of biomass as compared to inoculation with *Trichoderma* than in its absence. With respect to PSPT, it remains that the largest differences between treatments are seen at inoculant concentrations of 7 x 10^4 spores mL^{-1}, in which the highest values generated in the inoculation treatments were generated with the TRIC13 strain.

In addition to the P value ($P \leq 0.05$), it is notable that the Tukey test that was made identified differences between the median values of the PST, NFr , and PSFr plant variables for some dosis levels. Perhaps another experiment in which these interactions are evaluated in higher numbers will identify these tendencies with

greater precision.

Discussion

The results of the present investigation confirm the hypothesis made, indicating that the biometric response of *Phaseolus vulgaris* L is a function of the strain of *Trichoderma* spp. and the concentration in which it is inoculated in the plant, which is to say, there is a differential strain effect.

Experiment 1. In vitro germination of seeds

Various strains of *Trichoderma* spp., have a lithic action on the episperm of the seed and also solubilize nutrients through the excretion of organic acids and produce specific growth inductors that accelerate the development of primary meristematic tissues in the embrion; which augment the germination, volume, height, and mass of the plant (Moity 1982; Harman 2006; Hernández *et al.,* 2011 Samuels *et al.,* 2006; Shoresh and Harman 2008a, b). These mechanisms and their effects explain the values generated in the TRIC7 10^8, TRIC11 10^8, and TRIC 13^8 treatments, which showed the highest germination percentages (PG), with values of 93.1%, 91.1%, and 91.7%, respectively. There were statistically significant differences ($P \leq 0.05$) with respect to the control treatment. The results generated show a biopromoter effect with a growth function, as at higher concentrations of inoculate there are higher PG values.

Similar results were reported by Castro and Rivillas (2005), who inoculated coffee seeds with *T. harzianum* and obtained a 90% rate of germination, as compared to 70% in the control treatment. Cubillos-Hinojosa *et al.,* (2009) also reported in their investigations that there was a greater germination stimulation effect when passion fruit seeds were inoculated with *T. harzianum*, (93.3%) in relation to the control treatment. The results obtained with strains of *Trichoderma* spp. agree with investigations that report the production of metabolites that, when liberated in the growth medium, stimulate plant germination and development (Altomare *et al.,* 1999; Cubillos-Hinojosa *et al.,* 2009; Valencia *et al.,* 2005).

In all treatments, IVG was higher with respect to the control treatment. It was also found that at higher inoculant concentrations there were higher germination speeds; data that is consistent with that reported by Cubillos-Hinojosa *et al.,* (2009) in their experimets with cucumber. It was also found that the velocity of bean seed germination was directly proportional to inoculant concentration. This was the case when, in treatments inoculated with TRIC7, TRIC11, and TRIC13 at a concentration of 7 x 10^8 spores mL^{-1} , a larger effect on the speed of seed germination was found. This fact can be attributed to higher fungal populations on the seed cover and higher degradation of the seed episperm, thus stimulating the development of primary meristematic tissues and eventually the germination of the embrion (Moity 1982; Harman 2006; Hernández *et al.,* 2011 Samuels *et al.,* 2006; Shoresh and Harman 2008a, b). In all treatments, the MTG decreased: the selection of strain and concentration of inoculant were crucial in diminishing the MTG in bean seeds. Concordantly with Besnard and Davet (1993) and Cubillos-Hinojosa *et al.,* (2009), passion fruit and cucumber seeds, respectively, inoculated with *T. harzianum* germinated an average of two days before those that were not inoculated.

Experiment 2. Evaluation of bean plants at the greenhouse level

Trichoderma spp., inoculated in adequate quantities, stimulates germination, growth, and root development, which, in turn, promotes the assimilation of water and nutrients (Hernández *et al.,* 2011; Shoresh and Harman 2008b; Vinale *et al.,* 2008; Harman 2006; Cupull *et al.,* 2003). It follows from the above that bean plants inoculated with a fungal concentration of $7x10^4$ showed better root development, height, and biomass than treatments inoculated with higher concentrations.

Similarly, in their 2011 study, Hernández *et al.,* inoculated corn plants with *T. harzianum,* and found that inoculations with spores above $7x10^4$ spores mL^{-1} diminished the development of the plant. This effect may be due to phytotoxins that some strains of

Trichoderma spp. produce; when they are increased, the competition for nutrients and space show antagonism through competition and antibiosis (Shoresh and Harman 2008b; Harman 2006; Samuels *et al.,* 2006). Thus, $7x10^4$ spores mL^{-1} can be established as the optimal concentration for the inoculation of beans in order to generate adequate biopromotion.

Other investigations that reported important increases in the growth of seedlings when they were inoculated with specific concentrations of *T. harzianum* were done by Dandurand and Knudsen (1993), who saw an increase in bean biomass; Besnard and Davet (1993), who saw an increase biomass in cucumber; Windham *et al.,* (1986), whose apple seedlings were larger and more vigorous; Zambrano (1989), who saw an increase in tomato biomass; Börkman *et al.,* (1998), with better growth in the root systems of corn plants; and Cubillos-Hinojosa *et al.,* (2009), with better growth in passion fruit plants.

Also, it can be inferred that the beneficial results of *Trichoderma* spp., observed on the growth of bean plants evaluated in the present study are due to the interaction of mechanisms in which the capacity to phosphodissolve through the excretion of organic acids or their conjugal bases and the capacity to produce IAA (Cubillos-Hinojosa *et al.,* 2009; Valencia *et al.,* 2005), substances that favor the lengthening of roots, permit the better capture of nutrients in the soil on the part of the plant (Vera *et al.,* 2002 and Godes 2007). The above contributes to better vegetal nutrition.

Finally, an added value to the utilization of these microorganisms in agriculture is their potential to also act as biological controllers of soil phytopathogens, which has been reported in different studies (Torres-Rubio *et al.,* 2000; Rodríguez and Fraga 1999; Altamore *et al.,* 1999; Kloepper 1993), which is why different isolates have been utilized as active ingredients of different commercial bioproducts for agriculture in Colombia (Cubillos-Hinojosa *et al.,* 2009; Moreno *et al.,* 2007; Vera *et al.,* 2002). From the above, it can be inferred that the presence of phosphate solubilizing and IAA producing strains of *Trichoderma* spp. associated with plants may initiate similar effects and be used as inoculates in agricultural systems (Useche 2003; Rodríguez and Rubiano 2002; Cepeda and Gamboa 2001).

The presence and vegetal growth promoting activity of phosphate solubilizing and IAA producing strains of *Trichoderma* spp. is summarily important, as it offers plants the possibility to simultaneously achieve better adaptation to this phosphate-limited ecosystem through the help of assimilable forms of phosphorous that favor efficiency in the uptake of nutrients, thus promoting greater length and proliferation of roots (Villarroel 2009; Ocampo *et al.,* 2012; Valero 2003; Martínez and Martínez 2000).

The results of this study add credence to the additive hypothesis (Bashan *et al.,,* 1993), which states that microorganisms that promote vegetal growth act through the summation of different mechanisms that operate simultaneously or alternatively when they are induced under favorable environmental conditions that stimulate vegetal growth. The association of plants with microorganisms that act through various mechanisms in an integrated manner, such as phosphate solubilizers, may be an important mechanism of adaptation for the ecological success of both the plants and the microorganisms, and at the same time represent a valuable germplasm bank for agroecological and biotechnological applications.

Conclusion

In addition to the bioprotective effect reported against bean phytopathogens on the part of native strains of *Trichoderma,* there is also a stimulative effect on the *in* vitro germination of bean seeds. Although each strain and concentration of inoculate produces differential effects, its inoculation increases the velocity and germination percentage and decreases the average germination time. Also, the strains of *Trichoderma* that were evaluated significantly promote the development of plants,

generating important increases in all the evaluated variables, with total biomass and fruit dry mass standing out. The inoculation with native strains of *Trichoderma* spp. indicates that the formulation of local bioinputs generates better adaptation to the edaphoclimatic conditions of an ecosystem. After the quantity of available phosphorous and the P absorption capacity of a soil, the inoculation with HSP is summarily important, as it improves the adaptation of plants to ecosystems in which P is a limiting resource. Thus, advances in the development and evaluation of multifunctional bioinputs with biopromotive and biocontrol actions that favor the growth, development, and health of bean plants are very important.

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