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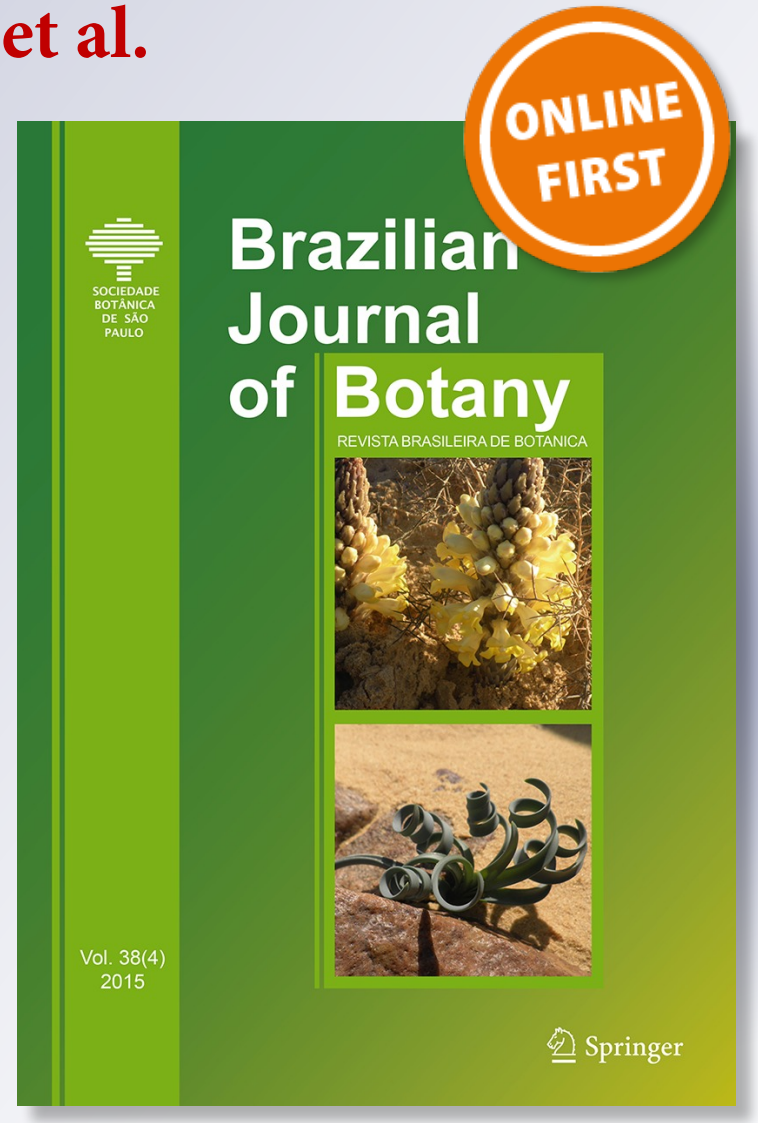

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Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis

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Abstract The aim of this study was to determine the efficiency of *Trichoderma* spp. in promoting the growth of cowpea plants. Strains were isolated from Tocantins savannah soils. Twenty-one isolates were evaluated in vitro for their ability to solubilize calcium phosphate and synthesize indole acetic acid (IAA). The soil samples were characterized physicochemically before planting. Eleven isolates were selected to evaluate the promotion of cowpea plant growth in the greenhouse in soil fertilized with phosphorite in 1.7-L pots. Then, at 32 and 45 days after planting (DAP), we determined the plant height (PH), root length (RL), dry matter of the aerial part (DMAP), root dry matter, total dry matter (TDM), relative efficiency (RE), and phosphate utilization efficiency (P-UEF) of the cowpea plants. The *Trichoderma* isolates showed a greater ability to synthesize IAA and solubilize phosphate than the controls. The best isolates for solubilizing phosphate were UFT 63, UFT 79, UFT 85, and UFT 201. The isolates UFT 25, UFT 79, UFT 110, and UFT 201 were more efficient in synthesizing IAA using tryptophan. The *Trichoderma* isolates exhibited P-UEF, especially isolates UFT 57, UFT 201, and UFT 204, with values of 430, 282, and 359 %, respectively, compared to controls. Consequently, the isolate UFT 201 (*T. asperelloides* GJS 04-217) showed greater potential as a growth promoter for cowpea, with a 38 cm PH, 29 cm RL, 4.5 g DMAP, and 5.8 g TDM at 45 DAP and a 256 % phosphorus content and 269 % RE compared to the control without inoculation.

Keywords Cowpea · Growth promoter · Indole acetic acid · Phosphate solubilization · *Trichoderma*

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Introduction

The cowpea (*Vigna unguiculata* L. Walp.) has been introduced into the savannah biome in Piauí, Maranhão, and Tocantins States, primarily due to its compatibility with the crop rotation system and the regional rainfall regime. In recent years, the cowpea crop in Tocantins State has been advancing at a rapid pace, with an increasing cultivated area. The culture possesses genetic variability, tolerance towards various edaphoclimatic conditions, a high productive potential, and an excellent nutritional value (Freire et al. 2005). However, the crop presents low average productivity. Therefore, studies that aim to generate technology to enable the planting of cowpea should be performed. Such advances would provide an economic alternative for farmers as a subsistence crop.

Fungi of the genus *Trichoderma* have great economic importance for agriculture and act as disease control agents for various crops, growth promoters in plants, and disease resistance inducers (Brotman et al. 2010; Machado et al. 2012; Oliveira et al. 2012). These fungal species have received attention from researchers due to their versatile action, and ability to produce antifungal substances and degrade the cell walls of other fungi. In addition, these species exhibit several survival strategies, making the natural environment highly competitive and ensuring that the rhizosphere has a great proliferation capacity (Louzada et al. 2009).

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Some mechanisms used by *Trichoderma* spp. have been highlighted: the promotion of plant growth, the production of phytohormones, such as indole acetic acid (IAA; Machado et al. 2011; Silva et al. 2012), and phosphate solubilization (Kapri and Tewari 2010). The biological control performed by the pathogen, particularly the competition for nutrients, the production of antibiotics, and induced resistance to diseases, such as the ability to remove pathogens, must also be mentioned (Silva et al. 2011; Gava and Menezes 2012).

However, considering applications in the food industry, with relatively low productivity, the cowpea crop is of great importance in the development of inoculants using native fungi of savannah regions. The ability of *Trichoderma* fungi to promote plant growth and increased productivity has been investigated for use as an inoculant, with positive responses.

Therefore, the study of the efficiency and capacity of *Trichoderma* to solubilize phosphate, synthesize IAA, and control diseases can contribute to the selection of potentially useful strains that can provide added phosphorus (P) to low-solubility sources and support their subsequent use in agriculture. Thus, this work aimed to select isolates of *Trichoderma* spp. from Tocantins savannah soils and evaluate their efficiency as cowpea growth promoters in the greenhouse.

Materials and methods

Isolation and preparation of strains

Twenty-one isolates of *Trichoderma* spp. from soil samples collected in areas at the experimental station of the Universidade Federal do Tocantins, Campus Gurupi (11°43′45″ S and 49°04′07″ W, 300 m average altitude), and in lowland areas of the municipality of Lagoa da Confusão, TO (10°47′37″ S and 49°37′25″ W, 200 m average altitude), were analyzed. The samples were collected at a depth of 0–10 cm of the soil profile in areas used for annual crops. The soil samples were deposited directly into Petri dishes (9 cm in diameter). The employed isolation method was the direct plating method, with three replicates per sample in PDA medium (200 g L^{-1} of potato, 20 g L^{-1} of dextrose, and 15 g L^{-1} of agar) supplemented with Terramicina®—Pfizer (100 mg L^{-1}) (0.1 g L^{-1}) to inhibit bacterial growth. The plates were incubated for 7 days in a biochemical oxygen demand growth chamber at 25 ± 2 °C, with a photoperiod of 12 h (Dianese et al. 2012). The strains were selected according to the characteristics of colonies of *Trichoderma*, including aggressive growth on PDA culture medium and a green color. Then, the preliminary identification was made based on morphological characteristics, especially reproduction characteristics, as described in the relevant literature, with the aid of an optical microscope (Barnett and Hunter 1998).

Soluble phosphate concentration analysis

For the phosphate solubilization assay, *Trichoderma* spp. isolates were initially incubated on PDA medium (200 g L^{-1} of potato, 20 g L^{-1} of dextrose, and 15 g L^{-1} of agar) at 25 ± 2 °C for 7 days. From these colonies, disks with a diameter of approximately 8.0 mm that contained mycelium and spores were removed. The disks were transferred to 250-mL Erlenmeyer flasks in modified NBRIP medium (Nautiyal 1999). The medium contained the following ingredients (g L^{-1}, Cromoline: Brazil): glucose, 10.0; $MgCl_2{\cdot}6H_2O$, 5.0; $MgSO_4{\cdot}7H_2O$, 0.25; KCl, 0.2; and $(NH_4)_2SO_4$, 0.1, with the addition of 50 mL of K_2HPO_4 (10 % w/v) and 100 mL of $CaCl_2$ (10 % w/v) to precipitate insoluble calcium phosphate ($CaHPO_4$). The pH was adjusted to 7.0.

Quantitative estimation of phosphate solubilization was performed in triplicate in a completely randomized design. The incubation was conducted at 25 ± 2 °C in an orbital shaker at 150 rpm for 8 days. For the determination of soluble P concentrations, the colorimetric method proposed by Murphy and Riley (1962) was used, using ammonium molybdate solution acidified with ascorbic acid. The soluble P contained in the treatments was subtracted from the sample contained in the control (culture medium with phosphate and without inoculum). For evaluations, we used one portion of the reagent described by Murphy and Riley (1962), 0.5 mL of the filtered sample, and 5 mL of distilled water for each sample. After 20 min of reaction, the soluble P was quantified in a spectrophotometer at a wavelength of 725 nm. The standard curve for the quantification of soluble P was generated using monobasic potassium phosphate (KH_2PO_4), and the calculated concentrations were presented in μg mL^{-1}.

Quantification of indole acetic acid production

For the in vitro production of IAA by *Trichoderma* spp., isolates were selected and grown in PDA medium, as described for the evaluation of phosphate solubilization ability. After 7 days of culture, 8.0-mm-diameter disks containing the fungal spores and mycelium were transferred to 250-mL Erlenmeyer flasks containing 50 mL of PD liquid medium (200 g L^{-1} of potato, 20 g L^{-1} of dextrose) in the presence and absence (control) of L-tryptophan. The concentration of L-tryptophan used was

100 mg L^{-1}, with three replicates per treatment (isolated) in a completely randomized design.

After 8 days of growth in an orbital shaker (150 rpm) at 26 ± 2 °C, the mycelial mass was separated by centrifugation at 12,000 rpm for 15 min. Colorimetric analysis of IAA was performed according to the procedure described by Gordon and Weber (1951), in which one portion of Salkowski reagent [$FeCl_3$ 0.5 mol L^{-1} + $HClO_4$ (35 %)] and two portions of the supernatant obtained from each isolate were used. After qualitative evidence of the presence of IAA (pink color after 25 min of reaction at 28 °C in the dark) was obtained, the phytohormone was quantified in a spectrophotometer at 530 nm. The concentrations were calculated based on a standard curve with known concentrations of IAA (3-IAA, $C_{10}H_9NO_2$, Vertec) in synthetic hormone form (0–100 μg L^{-1}). The absorbance readings were used to calculate the concentrations of IAA in the samples.

Molecular identification of isolates

To evaluate cowpea growth promotion ability, we selected 11 isolates of *Trichoderma*. The strain *Trichoderma harzianum* CIB T44 was used as a model strain for in vitro comparisons of growth potential and was obtained from Instituto Biológico de São Paulo (IB). The strains were tested for their phosphate solubilization capacity and for IAA production equal or superior to that of the model strain *T. harzianum* CIB T44. DNA extraction, amplification, and sequencing were performed according to the procedures described by Colonia and Chagas (2014). The strains were characterized at molecular level by sequencing the ITS regions (translation elongation factor) and identified using the access codes in GenBank (Table 1). The strains were identified by the Instituto Biológico de São Paulo (IB).

Prior inoculum for in vitro growth promotion assay

Polypropylene bags containing 300 g of commercial rice with 300 mL of distilled water were autoclaved at 121 °C for 1 h. After cooling, six fungal disks of 5 mm in diameter were inoculated. After 7 days of incubation, 30 g samples were collected from each bag of rice colonized by *Trichoderma* for subsequent inoculation of the soil.

The concentration of *Trichoderma* spp. used in the experiment was determined by quantifying the number of conidia. One gram of colonized rice was washed in 10 mL of sterile water, followed by stirring for 1 min and subsequent counting in a Neubauer chamber using an optical microscope. Concentrations of 1×10^9 conidia g^{-1} of colonized rice were used in the experiments. In the control, the substrate was added to rice without *Trichoderma* spp.

Physicochemical characterization of soil samples

Prior to planting, a composite soil sample was collected. Physical and chemical characterization of the sample was performed. The obtained values were as follows: 1.7 $cmol_c$ dm^{-3} Ca, 0.6 $cmol_c.dm^{-3}$ Mg, 17.4 $cmol_c.dm^{-3}$ K, 1.7 mg dm^{-3} P, 0.07 $cmol_c.dm^{-3}$ Al, 7.4 $cmol_c.dm^{-3}$ CTC, 19.7 $cmol_c.dm^{-3}$ SB, 39 % V, pH 5.4 in water, 1.0 % organic matter, and texture 72.3, 8.2, and 19.5 % sand, silt, and clay, respectively. The chemical properties of soil were determined at depth 0–20 cm as follows: pH in water, ratio 1:2.5; P and K, extractor Mehich 1, Al^{3+}, Ca^{2+}, and Mg^{2+}, extractor KCl (1 mol L^{-1}), H + Al, extractor SMP, BSE, basic sum exchangeable; CEC, cation exchange capacity at pH 7.0; *V*, base saturation index; and SOM, soil organic matter (oxidação: $Na_2Cr_2O_7$ 4 N + H_2SO_4 10 N) (Claessen et al. 1997).

Table 1 Identification and sequence analysis of the *Trichoderma* strains used in this study

Isolates	Species identification	Accession numbers	References
UFT 25	*T. harzianum* CIB T131	EU279988	Hoyos-Carvajal et al. (2009)
UFT 37	*T. pinnatum* GJS 02-120	JN175572	Druzhinina et al. (2012)
UFT 57	*T. virens* CIB T147	EU280060	Hoyos-Carvajal et al. (2009)
UFT 63	*T. virens* CIB T147	EU280060	Hoyos-Carvajal et al. (2009)
UFT 76	*T. harzianum* DAOM 167671	AY605783	Druzhinina et al. (2004)
UFT 79	*T. harzianum* DAOM 167671	AY605783	Druzhinina et al. (2004)
UFT 85	*T. harzianum* CIB T23	EU279989	Hoyos-Carvajal et al. (2009)
UFT 201	*T. asperelloides* GJS 04-217	DQ381958	Samuels et al. (2010)
UFT 202	*T. harzianum* CIB T23	EU279989	Hoyos-Carvajal et al. (2009)
UFT 204	*T. longibrachiatum* DAOM 167674	EU280046	Hoyos-Carvajal et al. (2009)
UFT 205	*T. asperelloides* GJS 04-217	DQ381958	Samuels et al. (2010)
Model strain	*T. harzianum* CIB T44	EU279989	Hoyos-Carvajal et al. (2009)

Evaluation of cowpea growth promotion in the greenhouse

The *Trichoderma* isolates were mixed with sieved soil. The soil was supplemented with insoluble phosphorite (adherent) at a dose of 100 mg kg^{-1} of soil to overcome P deficiency. The phosphate concentrate used were the Angico and the Galvani concentrates (fertilizer industry: Luiz Eduardo Magalhães, BA) (32 % total P_2O_5 and 2 % P_2O_5 soluble in citric acid), solubilized in citric acid solution of 20 g L^{-1} with agitation. Measurements of P were made using the spectrophotometric molybdovanadate-phosphoric acid method (Claessen et al. 1997).

The soil mixtures containing phosphorite and *Trichoderma* were placed individually in 1.7-L pots for 7 days for further planting of cowpea. During planting, six seeds of cowpea type Fradinho were placed in each pot. Five days after germination, thinning was performed and two plants were allowed in each pot. Inoculation with rhizobia (*Bradyrhizobium* sp. strain SEMIA 6462) at a concentration of 3×10^9 colony-forming units mL^{-1} was performed 1 h before planting. The inoculation rate was 500 mL of inoculum in 50 kg of seeds in order to achieve biological nitrogen fixation.

At 32 and 45 days after planting (DAP), the following growth variables were determined: plant height (PH), root length (RL), dry matter of the aerial part (DMAP), root dry matter (RDM), and total dry matter (TDM). At 45 days, the P content in the aerial part was determined (Claessen et al. 1997).

In the evaluation performed at 45 DAP, the number of nodes (NN) and the dry matter of the nodules (DMN) were determined by drying in an oven at 65 °C for 3 days. The determination of NN was conducted to investigate the possible effects of *Trichoderma* inoculation related to *Bradyrhizobium* sp. (strain SEMIA 6462) inoculated into cowpea seeds.

The relative efficiency (RE) of each treatment was determined using the second evaluation DMAP (Bergensen et al. 1971). The phosphate utilization efficiency (P-UEF) was determined using the aerial part P content (Siddiqi and Glass 1981). RE and P-UEF were calculated using the following equations:

$$\mathrm{RE} = \frac{\text{DMAP inoculated with isolates}}{\text{DMAP without inoculation}} \cdot 100$$

$$\text{P-UEF} = \frac{\mathrm{DMAP}^2}{\text{Nutrient content}}.$$

Statistical analysis

The experimental design was completely randomized, with 13 treatments (11 strains, the model strain of *T. harzianum* CIB T44, and a control without inoculation) with six replicates each. Data from different experiments were subjected to analysis of variance. To determine significance, means were compared in post hoc analysis using Scott–Knott's test at 5 % probability. The statistical program used was ASSISTAT version 7.6 beta.

Results

Calcium phosphate solubilization

Trichoderma spp. UFT 63 (24.76 μg mL^{-1}), UFT 79 (24.36 μg mL^{-1}), UFT 85 (25.47 μg mL^{-1}), and UFT 201 (24.76 μg mL^{-1}) presented a stronger phosphate solubilization capability ($p < 0.05$) compared to the other strains and the model strain (Table 2). These isolates showed, on average, a 20 % higher percentage of solubilization than the model strain *T. harzianum* CIB T44.

In relation to the pH, a reduction in the culture medium of most of the *Trichoderma* isolates and the model strain was observed in liquid medium with calcium phosphate (Table 2).

The phosphate solubilization capability of microorganisms can be related to the acidification of the culture medium due to the decrease in pH after the release of organic acids into the growth medium. pH reduction in fungal culture medium using *Aspergillus niger* was also observed by Vassilev et al. (2006).

However, in an experiment using *Trichoderma* spp., Kapri and Tewari (2010) concluded that although there is a reduction of the pH in individual cultures after 48 h and the value subsequently remains constant, the concentrations of soluble phosphate continue to increase after 48 h. This finding clearly suggests that the pH reduction is not the only factor involved in phosphate solubilization.

Although phosphate solubilization capability relates to the production of acids or the reduction of pH by microorganisms, these factors are not always correlated with the amount of soluble phosphate produced (Stamford and Nahas 2010). In addition to these factors, the fact that fungal growth is another important factor for phosphate solubilization should be considered (Barroso et al. 2006). In natural environments, such as soil, microorganisms tend to absorb the nutrients that are necessary for growth. According to Nahas (2007), the availability of these nutrients has a great influence on the phosphate solubilization ability in the soil. Consequently, the ability to produce acids and decrease the pH depends on the sources of C, N, and P.

Indole acetic acid synthesis

All of the *Trichoderma* isolates used in this study were able to produce IAA in BD culture medium supplemented or not

Table 2 Calcium phosphate solubilization (10 g L^{-1}) and indole acetic acid synthesis by *Trichoderma* spp. in the presence and absence of L-tryptophan

Isolates	Calcium phosphate solubilization			Indole acetic acid synthesis (μg mL^{-1})		
	μg mL^{-1}	*S* (%)[a]	pH	Without L-T	With L-T	IAA (%)[b]
UFT 25	12.6 d	62.0	4.7	4.4 aA	4.4 aA	176
UFT 28	15.6 c	76.6	3.4	1.1 fB	1.5 eA	60
UFT 32	15.3 c	75.3	4.2	1.6 eA	1.6 eA	64
UFT 35	8.1 e	39.9	6.1	1.3 fA	1.3 eA	52
UFT 37	20.4 b	100.1	6.1	4.3 aA	3.3 bB	132
UFT 57	9.0 e	44.4	6.2	2.0 dB	3.2 bA	128
UFT 63	24.8 a	121.7	5.4	1.5 eB	2.9 bA	116
UFT 76	20.4 b	100.4	5.6	1.5 eB	2.7 cA	108
UFT 78	19.7 b	97.0	5.8	1.0 fA	1.3 eA	52
UFT 79	24.4 a	119.8	4.4	3.6 bA	3.7 aA	148
UFT 85	25.5 a	125.2	5.4	1.3 fA	1.5 eA	60
UFT 87	18.5 b	91.2	5.5	1.7 eB	2.1 dA	84
UFT 92	8.9 e	43.8	6.1	1.4 fB	2.1 dA	84
UFT 102	9.2 e	45.4	5.1	1.9 dB	2.5 cA	100
UFT 104	8.6 e	42.3	5.4	2.2 dA	2.3 dA	92
UFT 110	12.4 d	61.0	4.5	2.9 cA	3.9 aA	156
UFT 111	16.8 c	81.9	5.8	1.4 dA	1.6 eA	64
UFT 201	24.8 a	121.7	5.4	2.6 cB	3.9 aA	156
UFT 202	20.4 b	100.2	5.3	2.6 cA	2.7 cA	108
UFT 204	20.7 b	101.9	5.5	2.2 dA	2.7 eA	108
UFT 205	21.8 b	107.2	5.5	2.7 cA	2.9 cA	116
T. harzianum CIB T44	20.3 b	100	4.5	1.9 dB	2.5 cA	100
Control	0.4 f	–	6.1	0.2 gA	0.2 hA	–
CV (%)	12.9	–		11.7	10.7	

Lowercase letters compare isolates and capital letters compare treatments. Different letters indicate statistically significant differences, as indicated by Scott–Knott's test at 5 % probability

S solubilization, *L-T* L-tryptophan, *IAA* indole acetic acid, *CV* coefficient of variation

[a] Phosphate solubilization percentage compared to the *T. harzianum* CIB T44 model strain

[b] Percentage calculated in the presence of L-tryptophan in comparison to the model *T. harzianum* CIB T44

supplemented with L-tryptophan (Table 2). For most of the isolates, IAA synthesis was greater in the presence of L-tryptophan. For isolates UFT 25 (4.4 μg mL^{-1}), UFT 79 (3.7 μg mL^{-1}), UFT 110 (3.9 μg mL^{-1}), and UFT 201 (3.9 μg mL^{-1}), we observed significantly higher values ($p < 0.05$) compared to other isolates grown in the presence of L-tryptophan. Considering these IAA concentrations as percentages of the values obtained for the model strain *T. harzianum* CIB T44 (% IAA) after 8 days of growth, these isolates showed higher values, with increases ranging from 48 to 76 % on average.

Growth analysis in the greenhouse

Regarding the inoculation of *Trichoderma* into cowpea seeds in the greenhouse, the isolate UFT 201 showed superior results ($p < 0.05$) for all variables at 32 DAP (Table 3). The treatments resulted in no significant difference in RL at 32 DAP. With respect to PH, nine isolates and the model strain were significantly higher ($p < 0.05$) compared to the control at 32 DAP.

With respect to DMAP and TDM, most isolates showed an increase compared to the control in cowpea plants at 32 DAP (Table 3). This effect was probably caused by the dissolution of insoluble phosphorite in the soil. Only the isolate UFT 76 for the DMAP and TDM variables and the isolate UFT 202 for the TDM variable failed to achieve significantly higher values than the control.

At 45 DAP, the isolates UFT 37, UFT 63, UFT 201, and UFT 204 were superior to the other treatments with respect to at least three growth variables. Significant correlations were observed between the variables PH × DMAP (0.743), PH × TDM (0.771), and DMAP × TDM (0.952). The results indicate that the growth of cowpea plants may also have been enhanced significantly by the inoculation of these *Trichoderma* isolates in the presence of phosphorite.

Table 3 Growth variables evaluated at 32 and 45 days after planting of cowpeas inoculated with *Trichoderma* spp.

Isolates	PH (cm)	RL (cm)	DMAP (g)	RDM (g)	TDM (g)	NN	DMN (g)
32 DAP							
UFT 25	31.7 a	25.7 a	1.7 b	0.7 c	2.4 c	–	–
UFT 37	31.7 a	29.7 a	1.8 b	0.6 c	2.4 c	–	–
UFT 57	29.3 a	25.0 a	1.5 c	0.7 c	2.2 c	–	–
UFT 63	33.7 a	26.3 a	2.2 a	1.0 b	3.2 b	–	–
UFT 76	26.0 b	32.7 a	1.1 d	0.6 c	1.7 d	–	–
UFT 79	31.0 a	32.7 a	1.7 b	0.6 c	2.3 c	–	–
UFT 85	28.0 b	26.7 a	1.5 c	0.8 c	2.3 c	–	–
UFT 201	35.0 a	29.3 a	2.3 a	1.3 a	3.6 a	–	–
UFT 202	30.3 a	35.7 a	1.3 c	0.6 c	1.9 d	–	–
UFT 204	33.0 a	31.3 a	1.9 b	0.8 c	2.7 c	–	–
UFT 205	30.7 a	30.7 a	1.7 b	0.6 c	2.3 c	–	–
T. harzianum CIB T44	33.0 a	27.0 a	1.8 b	0.6 c	2.4 c	–	–
Control	24.3 b	29.3 a	1.1 d	0.4 c	1.5 d	–	–
CV (%)	7.1	16.1	11.9	21.4	10.3	–	–
45 DAP							
UFT 25	30.7 b	27.7 b	2.3 b	1.6 a	3.9 b	16.7 c	27.7 c
UFT 37	33.7 a	27.3 b	2.4 b	1.7 a	4.1 a	15.3 c	13.3 d
UFT 57	30.3 b	27.3 b	3.8 a	1.1 c	4.9 a	12.3 c	12.0 d
UFT 63	32.7 b	30.3 a	2.6 b	1.9 a	4.5 a	29.0 b	16.0 d
UFT 76	28.7 b	25.0 c	1.4 c	1.0 c	2.4 c	10.0 c	17.0 d
UFT 79	31.0 b	27.7 b	2.3 b	1.3 b	3.6 b	22.3 b	53.3 b
UFT 85	31.0 b	25.0 c	1.9 c	1.1 c	3.0 c	27.7 b	27.0 c
UFT 201	38.0 a	29.0 a	4.5 a	1.3 b	5.8 a	42.3 a	141.7 a
UFT 202	32.0 b	26.3 c	2.3 b	1.3 b	3.6 b	16.3 c	36.0 c
UFT 204	35.0 a	27.3 b	3.8 a	1.2 c	5.0 a	25.3 b	31.0 c
UFT 205	31.7 b	32.0 a	2.7 b	1.0 c	3.7 b	15.0 c	12.0 d
T. harzianum CIB T44	32.0 b	25.0 c	2.8 b	1.5 b	4.3 a	13.0 c	18.0 d
Control	31.0 b	25.3 c	1.7 c	0.9 c	2.6 c	13.3 c	14.3 d
CV (%)	6.2	8.9	19.6	22.5	16.7	41.7	30.2

Different letters indicate statistically significant differences, as indicated by Scott–Knott's test at 5 % probability

PH plant height, *RL* root length, *DMAP* dry matter of the aerial part, *RDM* root dry matter, *TDM* total dry matter, *NN* number of nodes, *DMN* dry matter of nodules, *DAP* days after planting, *CV* coefficient of variation

Several studies reported the role of fungi from the genus *Trichoderma* as root growth promoters in the cultures of cucumber (Silva et al. 2011), cowpea (Oliveira et al. 2012), and rice (Asuming-Brempong 2013). These works highlighted the evidence of the potential of *Trichoderma* spp. as plant growth promoters in association with their phosphate-solubilizing capacity and IAA synthesis ability.

According to Brotman et al. (2010), *Trichoderma* spp. can promote up to 300 % increase in plant growth. The results of this study are in agreement with a study performed by Silva et al. (2011), in which the effect of *Trichoderma* on the growth of cucumber was evaluated and a significant increase compared to control without the inoculation of *Trichoderma* was observed.

With respect to NN and the DMN, the isolate UFT 201 was superior to the other isolates ($p < 0.05$; Table 3). There were significant correlations between the variables NN × DMN (0.789), DMN × DMAP (0.569), and TDM × NN (0.568). In general, different treatments yielded nodulation greater than or equal to the control treatment without the inoculation of *Trichoderma*, revealing no inhibitory effects of the inoculation of *Trichoderma* in rhizobia strains. Similar results were reported by Oliveira et al. (2012) after double inoculation of *Rhizobium* and *Trichoderma* in cowpea. Soares et al. (2006) found similar results, with higher values of NN and DMN than those observed after inoculating cowpea with rhizobia as a control.

The RE related to DMAP at 45 DAP after inoculation with *Trichoderma* spp. and after the control treatment revealed higher values ($p < 0.05$) for the treatments with inoculation by UFT 57, UFT 201, and UFT 204 isolates than for the other treatments without inoculation (Fig. 1).

Phosphorus evaluation in cowpea

With respect to P content, isolate UFT 201 was superior to the other isolates ($p < 0.05$), and this isolate and the other isolates UFT 37, UFT 63, UFT 205, UFT 25, UFT 204, UFT 85, UFT 202, UFT 57, and *T. harzianum* CIB T44 (model strain) were superior to the control that was not inoculated with *Trichoderma* (Table 4). The observed increase in the P content in relation to the control (P%) was calculated as an increase in the P content in the aerial part of the cowpea. In addition, there was an increase in most isolates compared to the control, with increases ranging from 13 to 156 %. There were significant correlations between the variables P × PH (0.851), P × RL (0.555), P × DMAP (0.651), P × TDM (0.705), P × NN (0.759), and P × DMN (0.689). With respect to P utilization efficiency (P-UEF), the highest values ($p < 0.05$) were found for the isolates UFT 57 and 204. The observed increase in the % P-UEF that was caused by the action of the isolates in comparison to the control values ranged from 45 to 330 % (Table 4).

Despite being a slightly soluble source and the only source of P used, the Angico phosphorite's (32 % of P_2O_5) low bioavailability may explain the phosphate solubilization ability of *Trichoderma* spp. in association with cowpea plants. These plants showed growth in the dry matter variables that are favored by the addition of this phosphate fertilizer (Table 3). This fact can be explained in terms of the aerial part P content in the cowpea inoculated with the isolate UFT 201 (7.69 g kg^{-1}; Table 4).

Table 4 Phosphorus assessment variables in cowpea plants inoculated by *Trichoderma* spp.

Isolates	P (g kg^{-1})	P%[a]	P-UEF	P-UEF%[b]
UFT 25	4.35 c	145	1.21 d	128
UFT 37	5.09 b	170	1.17 d	124
UFT 57	3.51 d	117	4.05 a	430
UFT 63	4.91 b	164	1.37 c	145
UFT 76	2.55 e	85	0.75 d	79
UFT 79	2.83 e	94	1.92 c	204
UFT 85	4.02 c	134	0.92 d	97
UFT 201	7.69 a	256	2.66 b	282
UFT 202	3.59 d	120	1.44 c	153
UFT 204	4.19 c	140	3.37 a	359
UFT 205	4.69 b	156	1.57 c	166
T. harzianum CIB T44	3.38 d	113	2.34 b	248
Control	3.0 e	100	0.94 d	100
CV (%)	11.5	–	12.1	–

Different letters indicate statistically significant differences, as indicated by Scott–Knott's test at 5 % probability

P phosphorus content, *P-UEF* phosphorus utilization efficiency, *CV* coefficient of variation

[a] Increased percentage of phosphorus content compared to the control

[b] Percentage increase in phosphorus utilization efficiency compared to the control

Discussion

When considering various phosphate sources, reactivity characteristics are determinants of efficiency. High-reactivity phosphates are rapidly available, favoring the absorption and utilization of P primarily by short-cycle crops. However, rock phosphate has a slower solubilization capacity, thus causing a gradual increase in P availability (Novais and Smyth 1999). The inoculation of *Trichoderma*

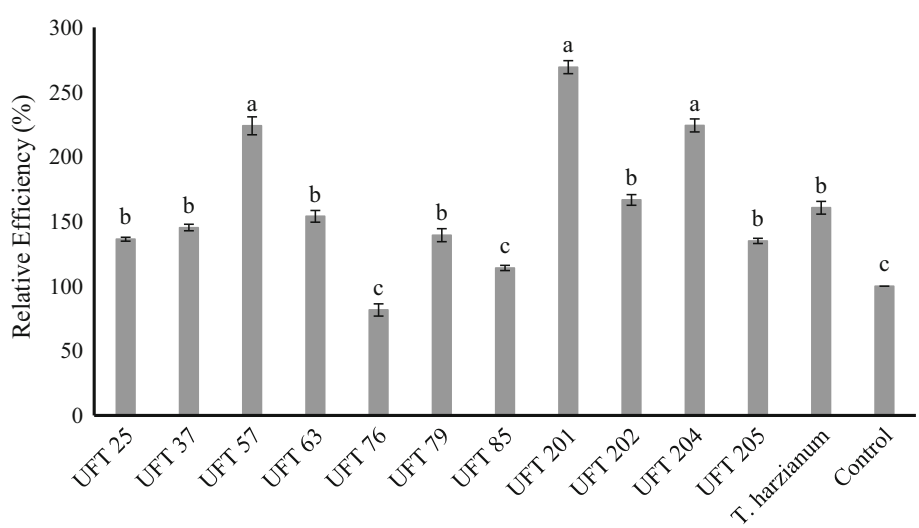

Fig. 1 Relative cowpea efficiency after inoculation with *Trichoderma* spp. in comparison to the control without inoculation. *Different letters* indicate statistically significant differences, as indicated by Scott–Knott's test at 5 % probability

isolates favored an increase in dry matter and P variables in the aerial part in comparison to the control treatment. This effect is explained by the phosphate solubilization ability of the biological control agents selected in this work.

Various factors can influence the phosphate solubilization capacity of *Trichoderma*, including the available carbon and nitrogen sources (Stamford and Nahas 2010), the type of cultivated plant (Grayston et al. 1997), and the type of phosphate that is solubilized (Barroso and Nahas 2005).

Similar results were also reported by Kapri and Tewari (2010), who inoculated *Trichoderma* into chickpea cultures and reported a phosphate solubilization capacity in vitro, with a significant effect on biomass production.

In the present study, we investigated the promotion of plant growth by *Trichoderma* spp. Significant increases in biomass related to DMAP and RDM were evident for some isolates. These results are consistent with those observed in other crops inoculated with specific strains of *Trichoderma* spp. According to Hoyos-Carvajal et al. (2009), the increase in biomass production related to growth hormones or analog production is another mechanism by which strains of *Trichoderma* spp. can improve the growth of plants. Several species of fungi have been reported to produce auxins. These fundamental hormones affect growth and development in fungal symbiotic interactions with plants (Machado et al. 2012).

The *Trichoderma* isolates exhibited IAA production and promoted the increase of cowpea plant biomass, indicating the presence of a relationship between hormones and biomass production. The higher accumulation of biomass in the cowpea plants may be related to hormone production or growth factors, to more efficient use of some nutrients, especially P, or to an increase in the availability and absorption of this nutrient by plants.

Thus, in addition to stimulating the synthesis of phytohormones, *Trichoderma* isolates can acidify the environment in which they settle through the secretion of organic acids, such as gluconic, fumaric, or citric acid (Gómez-Alarcón and Torre 1994). These acids are produced as a result of carbon source metabolism, particularly glucose, and the solubilization of phosphate, micronutrients, and cations, including iron, manganese, and magnesium. Thus, according to Ribeiro (2010), the addition of *Trichoderma* to soils with low levels of these cations could result in biofertilization via the solubilization of the available metals or the addition of slightly soluble natural phosphate as an alternative P supply in the soil, increasing biomass and crop productivity for crops such as cowpea.

The results of this study show the potential of *Trichoderma* species as plant growth promoters via their phosphate solubilization capacity and the synthesis of IAA. Current opinions on the use of these fungal species in commercial formulations designed as plant growth promoters are promising. The edaphoclimatic conditions are directly related to the biological activity efficiency of these fungi (Akrami et al. 2011). Therefore, the selection of *Trichoderma* isolates that originate from the regions where they will be used for the promotion of plant growth is important.

Conclusion

The *Trichoderma* isolates showed greater phosphate solubilization capacity and IAA synthesis ability than the control. The isolates UFT 63, UFT 79, UFT 85, and UFT 201 had higher calcium phosphate solubilization values. The UFT 25 and UFT 37 isolates showed a greater ability to synthesize IAA without the use of tryptophan, and the isolates UFT 25, UFT 79, UFT 110, and UFT 201 showed a greater ability to synthesize IAA with the use of tryptophan as an IAA precursor. The isolates showed potential as growth promoters for cowpea, especially isolates UFT 57, UFT 201, and UFT 204 with respect to DMAP, TDM, P content, and P utilization efficiency variables.

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