



## RESEARCH ARTICLE

### RICE GROWTH INFLUENCE BY *TRICHODERMA* SPP. WITH NATURAL PHOSPHATE FERTILIZATION UNDER GREENHOUSE CONDITIONS

Lillian França Borges Chagas, Brigitte. S. Orozco Colonia, Gil Rodrigues dos Santos,  
\*Gessiel Newton Scheidt, Augustus Caesar Franke Portella, Layssah Passos Soares  
and Aloisio Freitas Chagas Junior

<sup>1</sup>Department of Plant Production, Federal University of Tocantins (UFT), Gurupi, TO, Brazil.

<sup>2</sup>Bioprocess Engineering and Biotechnology Division, Federal University of Tocantins (UFT), Gurupi, TO, Brazil

<sup>3</sup>Phytopathology Laboratory, JCO Fertilizer, Barreiras, BA, Brazil

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#### ABSTRACT

The savannah soil is characterized by a lower fertility level. Thus, the study aimed to evaluate the effect of *Trichoderma* spp. inoculated in soil fertilized with natural phosphate in the growth of rice under greenhouse conditions. Twelve *Trichoderma* spp. isolates from savannah soil and one standard strain (*T. harzianum*) were used. The isolates were mixed to the soil fertilized with insoluble natural phosphate at a concentration of  $2 \times 10^8$  conidia per gram of colonized rice, at a dosage of 100 mg kg<sup>-1</sup> of soil, using pots with a capacity of 2.0 kg. Rice plants were evaluated for biomass production and phosphorus content. Then, UFT 25 and UFT 37 isolates were more efficient in rice growth and development, and showed higher phosphorus utilization efficiency. Most of the isolates showed to be directly involved in promoting the growth of this crop when compared to the uninoculated control, evidencing the potential of *Trichoderma* isolates as growth promoters of rice plants.

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#### INTRODUCTION

Phosphorus (P) is one of the most important elements for the plant growth and the limitation in its availability at the beginning of the vegetative cycle can result in restrictions on the development of the plant, thus, the plant does not recover even if that phosphorus is later supplied at optimal levels. However, the supply of phosphorus is essential from the beginning of plant growth (Grand *et al.*, 2001). However, tropical soils have a low concentration of soluble phosphorus ranging from 0.05 to 10 ppm, and more than 80% of phosphorus has no mobility, and neither is available for absorption in plants due to adsorption, precipitation or conversion in organic form (Holford, 1997). In order to alleviate this deficiency, large quantities of soluble fertilizers are commercially available. Although natural phosphates have lower agronomic efficiency due to low solubility, especially for annual crops, they can be an alternative to substitute these soluble fertilizers for the more gradual release, which reduces the phosphorus fixation process in these soils (Machado and Souza, 2012).

\*Corresponding author: Gessiel Newton Scheidt

Department of Plant Production, Federal University of Tocantins (UFT), Gurupi, TO, Brazil.

Many researchers have observed that a high proportion of phosphate solubilizing microorganisms especially bacteria, fungi and actinomycetes reside in the rhizosphere of plants and play an important role in the solubilization of bound phosphates, making them available to plants (Gravel *et al.*, 2007; Silva *et al.*, 2011). However, studies have reported that fungi have a greater ability to solubilize phosphate (Souchie *et al.*, 2007; Barroso and Nahas, 2008). The genus *Trichoderma* has been prominent in the phosphate solubilization studies among the fungi group (Kapri and Tewari, 2010; Chagas *et al.*, 2015). According to Fernández *et al.* (2008) tropical soils are normally acidic (pH 4 to 5) and have low availability of labile phosphorus, where the *Trichoderma* spp. isolates can be used as potential in phosphates solubilization in tropical soils. In addition, *Trichoderma* species are always associated with root colonization which often increases root growth and plant development, facilitating the absorption and utilization of nutrients, increasing resistance to abiotic stresses, and consequently the productivity of the crops. Several studies have shown *Trichoderma* species that promote these effects (Kapri and Tewari, 2010; Machado *et al.*, 2011; Hannan *et al.*, 2013; Chagas *et al.*, 2015; Chagas *et al.*, 2016), which stimulate the growth of several plant species and also protects against several pathogens. Therefore, the objective of this

study was to evaluate the effect of *Trichoderma* spp. Isolates, inoculated on natural phosphate fertilized soils in order to evaluate the rice growth in savannah biome under greenhouse conditions.

## MATERIALS AND METHODS

### *Trichoderma* isolates

Twelve *Trichoderma* isolates from Microbiology Laboratory Collection at Federal University of Tocantins (UFT), Gurupi Campus and a standard strain (*T. harzianum*) were used. These isolates were selected due to their phosphate solubilization capacity and indole acetic acid (IAA) synthesis. The strains were characterized by the sequencing of the TEF (translation elongation factor) and identified by access codes in GenBank and carried out by the Biological Institute of Sao Paulo. *Trichoderma harzianum* (CIB T44) was obtained from the Biological Institute of Sao Paulo.

### Physical-chemical characterization of soil

Before planting, a sample of soil was collected and the granulometric and chemical characterization was carried out at the Soil Laboratory of the Federal University of Tocantins, where the following values were found: 1.7 cmol<sub>c</sub>dm<sup>-3</sup>Ca, 0.6 cmol<sub>c</sub>dm<sup>-3</sup> Mg, 174 mg.dm<sup>-3</sup> K, 1.7 mgdm<sup>-3</sup> P, 0.07 cmol<sub>c</sub>dm<sup>-3</sup> Al, 7.4 cmol<sub>c</sub>dm<sup>-3</sup> CTC, 2.3 cmol<sub>c</sub>dm<sup>-3</sup> SB, 39% V, pH 5.4 in water, 1.0% organic matter, texture: 72.3, 8.2 and 19.5% sand, silt, and clay, respectively.

The chemical attributes at 0-20 cm depth were determined as follows: pH in water - ratio 1:2.5, P and K - extractor Mehlich 1, Al<sup>3+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> - extractor KCl (1 molL<sup>-1</sup>), 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 (oxidation: Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 4N + H<sub>2</sub>SO<sub>4</sub> 10N) (Embrapa, 2009).

**Table 1. GenBank access codes for *Trichoderma* spp. (TEF - translation elongation factor) used in this study**

Isolates	Species Identification	GenBank access	Reference
UFT 02	<i>T. harzianum</i> CIB T131	EU279988	Hoyos-Carvajal et al. (2009b)
UFT 25	<i>T. harzianum</i> CIB T131	EU279988	Hoyos-Carvajal et al. (2009b)
UFT 37	<i>T. pinnatum</i> GJS 02-120	JN175572	Druzhinina et al. (2012)
UFT 57	<i>T. virens</i> CIB T147	EU280060	Hoyos-Carvajal et al. (2009b)
UFT 63	<i>T. virens</i> CIB T147	EU280060	Hoyos-Carvajal et al. (2009b)
UFT 76	<i>T. harzianum</i> DAOM 167671	AY605783	Druzhinina et al. (2005)
UFT 79	<i>T. harzianum</i> DAOM 167671	AY605783	Druzhinina et al. (2005)
UFT 85	<i>T. harzianum</i> CIB T23	EU279989	Hoyos-Carvajal et al. (2009b)
UFT 205	<i>T. asperelloides</i> GJS 04-217	DQ381958	Samuels et al. (2010)
UFT 204	<i>T. longibrachiatum</i> DAOM 167674	EU280046	Hoyos-Carvajal et al. (2009b)
UFT 202	<i>T. harzianum</i> CIB T23	EU279989	Hoyos-Carvajal et al. (2009b)
UFT 201	<i>T. asperelloides</i> GJS 04-217	DQ381958	Samuels et al. (2010)
Standard strain	<i>T. harzianum</i> CIB T23	EU279989	Hoyos-Carvajal et al. (2009b)

### *Trichoderma* inoculation in greenhouse

The isolates were grown separately in PDA petri plates (200 g potato, 20 g dextrose, 15 g agar in 1000 ml water) and incubated at 25 ± 2 ° C for seven days with 12-hour photoperiod, time required for the growth of *Trichoderma* colonies. Polypropylene bags containing 300 g of commercial rice plus 300 mL of distilled water were autoclaved at 121 ° C for 1 hour and after cooling six 5 mm diameter discs of each isolate were placed separately constituted by mycelia and spores of *Trichoderma* in PDA medium. Then, the rice bags inoculated with the *Trichoderma* isolates were incubated in B.O.D incubator (Biochemical Oxygen Demand) at 25 ± 2 ° C for seven days with 12-hour photoperiod. Every two days, the rice was turned over substrate to facilitate gas exchange, breakage of mycelial aggregates and increase sporulation. After seven days of incubation, 30 g of each bag of rice colonized by *Trichoderma* were removed for subsequent classified as a dystrophic Red-yellow Latosol, with a medium texture.

The *Trichoderma* spp. concentration was determined by the serial dilution method and quantified as colony forming unit (CFU) per gram of colonized rice. The number of conidia was also quantified, where 1 g of colonized rice was washed in 10 mL of sterilized water, followed by shaking for 1 min, and counting the conidia in a Neubauer chamber using an optical microscope. Concentrations of 2 x 10<sup>8</sup> CFU per gram of colonized rice were used in the experiment. In the control, the rice was added to the substrate without *Trichoderma* spp. inoculation.

### Natural phosphate fertilization

The *Trichoderma* spp. isolates were mixed to the sieved soil. This phosphorus-deficient soil was supplemented with insoluble natural phosphate at a concentration of 100 mg.kg<sup>-1</sup> soil (65 kg de P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). The phosphate concentrate used was Angico, obtained from Galvani (Fertilizer Industry of Luiz Eduardo Magalhães, BA, Brazil), with 32% total content of P<sub>2</sub>O<sub>5</sub> extracted in HCl digestion, soluble in citric acid at 2%, and solubilized in 20 gL<sup>-1</sup> citric acid solution by stirring. The determinations were carried out by spectrophotometric method of molybdovanadophosphoric acid (Embrapa, 2009). The soil mixtures with the natural phosphate and *Trichoderma* isolates were placed individually in a 2.0 kg pot, remaining for seven days for later planting. The rice seeds were sown by adding six seeds per pot, thinning five days after germination, leaving two plants per pot.

### Biomass assays

The plants were harvested at 34 and 47 days after planting (DAP) for subsequent evaluations. Plant height (PH), root length (RL), dry mass of aerial part (DMAP), root dry mass (RDM) and total dry mass (TDM) were determined. The phosphorus content in aerial part was evaluated at 47 DAP (days after planting) (Embrapa, 2009). Thus, the relative efficiency (RE) was determined for each treatment using the biomass data, and calculated as follows: RE = (DMAP inoculated by isolates / DMAP without inoculation) x 100. Finally, the phosphorus utilization efficiency (P-UEF) was determined using the phosphorus content of aerial part in rice

plants and calculated by the following formula:  $P\text{-UEF} = \frac{[(\text{DMAP})^2 / (\text{Nutrient content})]}{}$  (Rodrigues *et al.*, 2003).

### Statistical analysis

The experimental design was completely randomized with 14 treatments and six replicates, where 13 were inoculated by *Trichoderma* and one control without inoculation. The data were submitted to analysis of variance by the F test, and the means of the treatments grouped by the Scott-Knott test at 1 or 5% probability using the statistical program Assistat (Silva, 2008).

DMAP, 2.37 to 2.54 g RDM, and 4.01 to 4.30 g TDM. Therefore, the values were over 250% higher than the standard strain (*T. harzianum* CIB T44) and more than 470% higher than the control for the total dry mass (TDM) variable. On the other hand, the relative efficiency (RE) correlates the aerial part biomass of treatments inoculated by *Trichoderma* spp. with the control without inoculation. Figure 1 shows higher values ( $p < 0.05$ ) for treatments with inoculation by UFT 25 and UFT 37, ranging from 410 to 440% RE, with an increase of more than 300% compared to *T. harzianum* CIB T44 and control.

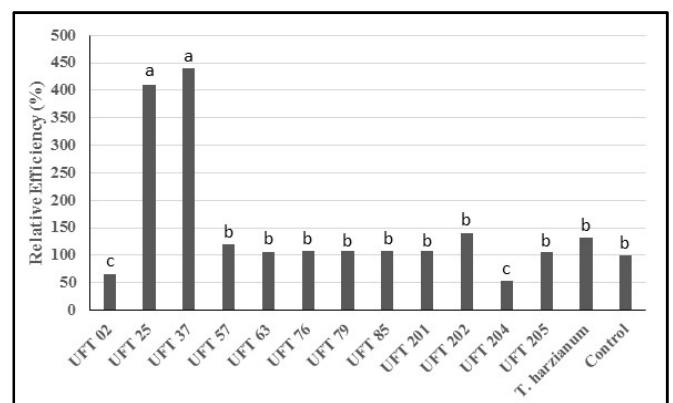
**Table 2. Mean values for plant height (PH), root length (RL), dry mass of aerial part (DMAP), root dry mass (RDM) and total dry mass (TDM) of rice in soil fertilized with natural phosphate and inoculated by *Trichoderma* strains**

Isolates	PH (cm)	RL (cm)	DMAP (g)	RDM (g)	TDM (g)
34 DAP					
UFT 02	23.7 b	27.0 b	0.15 d	0.27 b	0.42 b
UFT 25	48.3 a	38.3 a	1.15 a	1.71 a	2.86 a
UFT 37	53.0 a	33.0 a	1.18 a	1.51 a	2.69 a
UFT 57	23.0 b	33.3 a	0.11 d	0.18 b	0.28 b
UFT 63	25.0 b	37.0 a	0.14 d	0.25 b	0.39 b
UFT 76	44.0 a	30.7 b	1.11 a	1.49 a	2.60 a
UFT 79	25.3 b	36.7 a	0.16 d	0.40 b	0.56 b
UFT 85	25.3 b	34.3 a	0.15 d	0.27 b	0.42 b
UFT 201	21.0 b	25.3 b	0.09 d	0.16 b	0.25 b
UFT 202	31.3 b	27.3 b	0.21 d	0.34 b	0.55 b
UFT 204	20.7 b	26.7 b	0.12 d	0.15 b	0.27 b
UFT 205	27.3 b	29.0 b	0.18 d	0.28 b	0.46 b
<i>T. harzianum</i> CIB T44	24.0 b	21.7 b	0.17 d	0.19 b	0.36 b
Control	18.0 b	26.0 b	0.13 d	0.15 b	0.25 b
CV (%)	14.5	13.4	19.1	39.1	23.8
47 DAP					
UFT 02	28.3 b	22.7 a	0.26 b	0.80 b	1.06 b
UFT 25	46.3 a	22.9 a	1.64 a	2.37 a	4.01 a
UFT 37	52.3 a	22.3 a	1.76 a	2.54 a	4.30 a
UFT 57	37.7 b	22.5 a	0.48 b	0.84 b	1.32 b
UFT 63	32.3 b	22.0 a	0.42 b	0.96 b	1.38 b
UFT 76	33.7 b	22.5 a	0.43 b	0.89 b	1.32 b
UFT 79	32.3 b	22.1 c	0.43 b	1.12 b	1.55 b
UFT 85	36.3 b	16.3 c	0.43 b	0.88 b	1.31 b
UFT 201	33.0 b	22.0 a	0.43 b	0.42 b	0.85 b
UFT 202	39.7 b	18.0 b	0.56 b	0.78 b	1.34 b
UFT 204	25.7 b	22.1 a	0.21 b	0.48 b	0.69 b
UFT 205	32.0 b	22.0 a	0.42 b	1.25 b	1.67 b
<i>T. harzianum</i> CIB T44	37.3 b	22.4 a	0.53 b	1.06 b	1.59 b
Control	33.0 b	19.3 b	0.40 b	0.35 b	0.85 b
CV (%)	13.3	5.5	19.4	17.3	21.9

Averages followed by the same letter do not differ statistically by the Scott-Knott test at 5% significance. DAP days after planting. CV coefficient of variation.

## RESULTS

The influence of *Trichoderma* inoculation on rice in all attributes evaluated at 34 DAP (Table 2) allowed the plants growth, being statistically superior in the presence of natural phosphate by UFT 25, UFT 37 and UFT 76 isolates inoculation, in relation to others strains and the control. The only exception was for root length (RL) in isolate UFT 76, where no statistical difference was observed in relation to the control. The biomass values of these isolates ranged from 1.11 to 1.18 g DMAP, 1.49 to 1.71 g RDM, and 2.60 to 2.86 g TDM. In the case of total dry mass (TDM), the values were more than 700% higher than the standard strain (*T. harzianum* CIB T44) and more than 1000% higher than the control (Table 2). At 47 days after planting (DAP), the isolates UFT 25 and UFT 37 were higher ( $p < 0.05$ ) for plant height (PH), dry mass of aerial part (DMAP), root dry mass (RDM) and total dry mass (TDM) than the other isolates and control (Table 2). In these isolates was observed a variation from 1.64 to 1.76 g



**Figure 1. Relative efficiency of rice inoculated by *Trichoderma* spp. in relation to the control without inoculation (Means followed by the same lowercase letter do not differ by Scott-Knott's test at 5% probability)**

**Table 3. Mean values for phosphorus content in the aerial part (P) and phosphorus utilization efficiency (P-UEF) in rice inoculated by *Trichoderma* spp**

Isolates	P (gkg <sup>-1</sup> )	% P increase <sup>1</sup>	P-UEF	% P-UEF increase <sup>1</sup>
UFT 02	2.96 b	144	0.023 d	29
UFT 25	3.50 b	170	0.768 a	989
UFT 37	3.07 b	149	1.009 a	1299
UFT 57	2.02 c	98	0.114 b	147
UFT 63	2.68 c	130	0.066 c	85
UFT 76	3.21 b	156	0.058 c	74
UFT 79	2.57 c	125	0.072 c	93
UFT 85	4.52 a	219	0.041 c	53
UFT 201	2.27 c	110	0.081 c	105
UFT 202	2.72 c	132	0.115 b	148
UFT 204	2.99 b	145	0.015 d	19
UFT 205	2.10 c	102	0.084 c	108
<i>T. harzianum</i> CIB T44	2.31 c	112	0.122 b	157
Control	2.06 c	100	0.078 c	100
CV (%)	21.2	9.8	21.6	8.1

Averages followed by the same letter do not differ statistically by the Scott-Knott test at 5% significance. <sup>1</sup> Increase values determined in relation to the control. P phosphorus content. P-UEF phosphorus utilization efficiency. CV coefficient of variation.

The UFT 85 isolate was highlighted in the phosphorus content of the aerial part ( $p < 0.05$ ). Thus, followed by UFT 25, UFT 76, UFT 37, UFT 204 and UFT 02 isolates that were superior to the standard strain (*T. harzianum* CIB T44) and the control without inoculation (Table 3). There was an increase in the phosphorus content between the UFT 85 isolate and the other isolates higher than the control from 44 to 119%. The highest values ( $p < 0.05$ ) for phosphorus utilization efficiency (P-UEF) were found for the UFT 37 and UFT 25 isolates, followed by the standard strain (*T. harzianum* CIB T44) and the UFT 57 and UFT 202 isolates higher than the control (Table 3). The increase in the percentage of P-UEF between the isolates UFT 25 and UFT 37 and the other isolates were superior to the control from 47 to 1199%.

## DISCUSSION

According to Brotman et al. (2010), species of *Trichoderma* spp. can promote increases of up to 300% in plant growth. The results of this study corroborate the values reported by Silva et al. (2011), where they evaluated the effect of *Trichoderma* on cucumber growth and observed a significant increase in relation to the control without inoculation of *Trichoderma*. The increase observed in the DMAP, RDM and TDM variables among some isolates in relation to the control for the rice plants was probably due to the dissolution of the insoluble natural phosphate altered in the soil when compared to the uninoculated control. Even though the Angico natural phosphate (32% P<sub>2</sub>O<sub>5</sub>) used in the present study, a poorly soluble source resulted in the biomass growth of rice plants (Table 2).

This fact can also be explained as a function of the phosphorus contents of the aerial part of the rice for the isolate UFT 85 (4.51 gkg<sup>-1</sup>). Inoculation of some *Trichoderma* isolates, such as the UFT 85 isolates for the phosphorus content, and the UFT 25 and UFT 37 isolates for the P-UEF, significantly increased the phosphorus absorption of the culture. The ability of phosphate solubilization by soil microorganisms has been known for its influence on rice production (Sutaliya and Singh, 2005; Asuming-Brempong, 2013). The different sources of phosphate have reactivity characteristics that are determinant in relation to their efficiency. According to Bedin et al. (2003), high reactivity phosphates are more readily available, they aid

in the absorption and utilization of phosphorus mainly by short cycle crops. However, natural phosphates have slower solubilization and a gradual increase in phosphorus availability occurs (Novais and Smyth, 1999). Thus, inoculation by *Trichoderma* isolates may have favored the increase in DMAP, RDM and TDM and phosphorus content in the aerial part compared to the control treatment for their phosphate solubilization capacity. A number of factors may influence the phosphate solubilization capacity of *Trichoderma* spp., as the source of carbon and nitrogen available (Stamford and Nahas, 2010), the type of plant cultivated (Grayston et al., 1996), the type of solubilized phosphate (Barroso et al., 2005), among others, such as the presence of herbicides or insecticides (Reiset et al., 2008). In the present investigation, the promotion of plant growth by *Trichoderma* spp. isolates, the significant increase in aerial part biomass production and roots by UFT 25 and UFT 37 isolates was evident.

These results are in agreement with those observed in other agricultural crops inoculated with specific strains of *Trichoderma* spp. According to Hoyos-Carvajal et al. (2009a) the increase in biomass production due to inoculation by *Trichoderma* spp. may be related to the production of growth hormones or similar. Thus, *Trichoderma* isolates can acidify the environment where they are established by the secretion of organic acids, such as gluconic, citric or fumaric acids together with the synthesis or stimulation of phytohormone production, (Gómez-Alarcón and Torre, 1994). According to Harman et al. (2004), these acids are a result of carbon source metabolism, mainly glucose, and they are able to solubilize phosphates, micronutrients and cations, including iron, manganese and magnesium. Therefore, according to Benítez et al. (2004) and Ribeiro (2009), the addition of *Trichoderma* in cation-poor soils could result in biofertilization by the solubilization of the available metals or the addition of natural phosphates with low solubility as an alternative to phosphorus supplies in the soil, and the increase in biomass production and yield of crops such as rice.

## Conclusion

*Trichoderma* UFT 25 and UFT 37 isolates are more efficient in promoting rice crop growth and have been shown to be more efficient in the use of phosphorus by plants.

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