Revista Ciência Agronômica, v. 52, n. 4, e20207697, 2021
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Trichoderma in the promotion of growth and nutrition of dwarf cashew rootstock[1]

Trichoderma na promoção do crescimento e nutrição de porta-enxerto de cajueiro-anão

João Marcos Rodrigues dos Santos[2], Carlos Alberto Kenji Taniguchi[3], Christiana de Fátima Bruce da Silva[3], William Natale[4], Adriana Guirado Artur[2]

ABSTRACT - Inoculation of microorganisms in plants performs important functions related to plant growth and nutrition. The responses are based on hormonal stimulation and improved absorption and efficiency in the use of nutrients. Thus, the objective of this study was to evaluate the ability of *Trichoderma* strains (*T. longibrachiatum* and *T. asperellum*) to promote growth and improve the nutrition in dwarf cashew rootstock. The experiment was conducted in a shade net house with 50% of shading located in the Experimental Field of Pacajus, belonging to Embrapa Agroindústria Tropical, in Pacajus/CE, Brazil. The experimental design used was completely randomized, with three treatments and ten replicates. Each experimental unit consisted of a tube with a 288 cm^3 capacity containing one seedling of dwarf cashew. At sixty days (60) after germination, the plants were evaluated for biometric parameters, Dickson's quality index, and nutritional diagnosis of leaves. To verify the growth-promoting mechanisms, the strains were reisolated by indirect plating of roots. Nutrient accumulation in the leaves was not influenced by the application of the isolates; however, the seedlings were adequately supplied. The strains showed similarities in relation to growth-promotion mechanisms. The *Trichoderma longibrachiatum* strain promoted an increase in the growth, so its use as a supplement for the planting substrate is recommended.

Key words: *Anacardium occidentale*. 3-Indoleacetic Acid. Biostimulant. Siderophores. Solubilization of phosphates.

RESUMO - A inoculação de microrganismos em plantas desempenha funções importantes ligadas ao crescimento e a nutrição do vegetal. As respostas são baseadas na estimulação hormonal e na melhoria da absorção e eficiência no uso dos nutrientes. Assim, objetivou-se avaliar a capacidade de cepas de *Trichoderma* (*T. longibrachiatum* e *T. asperellum*) em promover o crescimento e melhorar a nutrição de porta-enxerto de cajueiro-anão. O experimento foi conduzido em telado com 50% de sombreamento, localizado no Campo Experimental de Pacajus, pertencente a Embrapa Agroindústria Tropical, em Pacajus/CE. O delineamento experimental utilizado foi inteiramente casualizado, com três tratamentos e dez repetições. Cada unidade experimental foi constituída por um tubete de 288 cm^3 de volume contendo uma muda de cajueiro-anão. Aos sessenta (60) dias após a germinação, as plantas foram avaliadas quanto aos parâmetros biométricos, o índice de qualidade de Dickson, e a diagnose nutricional das folhas. Para verificar os mecanismos promotores de crescimento, as cepas foram reisoladas pelo plaqueamento indireto de raízes. O acúmulo de nutrientes nas folhas não foi influenciado pela aplicação dos isolados, contudo, as mudas estavam supridas adequadamente. As cepas apresentaram semelhanças em relação aos mecanismos de promoção do crescimento. A cepa *Trichoderma longibrachiatum* promoveu aumento do crescimento do porta-enxerto, sendo, portanto, indicada a sua utilização como suplemento do substrato de plantio.

Palavras-chave: *Anacardium occidentale*. Ácido-3-Indolacético. Bioestimulante. Sideróforos. Solubilização de fosfatos.

DOI: 10.5935/1806-6690.20210053
Editor-in-Chief: Prof. Alek Sandro Dutra – alekdutra@ufc.br
*Author for correspondence
Received for publication 09/11/2020; approved on 27/04/2021
[1]Part of the Master's Dissertation of the first author
[2]Departamento de Ciências do Solo, Universidade Federal do Ceará/UFC, Fortaleza-CE, Brasil, joaorodriguesestagio.embrapa@gmail.com (ORCID ID 0000-0001-8543-5668); driguirado@yahoo.com.br (ORCID ID 0000-0001-6009-2750)
[3]Embrapa Agroindústria Tropical, Fortaleza-CE, Brasil, carlos.taniguchi@embrapa.br (ORCID ID 0000-0002-1280-8678), christiana.bruce@embrapa.br (ORCID ID 0000-0002-1794-8581)
[4]Departamento de Fitotecnia, Universidade Federal do Ceará/UFC, Fortaleza-CE, Brasil, 60356-000, natale@ufc.br (ORCID ID 0000-0001-9572-4463)

INTRODUCTION

Fruit growing is one of the segments of the Agrobusiness that have been increasing the most in Brazil in recent years. According to data from the Brazilian Association of Producers Exporters of Fruits and Derivatives (ASSOCIAÇÃO BRASILEIRA DE PRODUTORES EXPORTADORES DE FRUTAS E DERIVADOS, 2019), in 2019 about 980 tons of fruits were exported, which correspond to US$858 million injected into the national economy. Among the tropical fruit crops, cashew (*Anacardium occidentale* L.), native to the Brazilian territory, has shown great socioeconomic importance, mainly for the semiarid region, for generating jobs and income in the driest season of the year (SERRANO *et al.*, 2013).

The harvested area of cashew, which reached 758,000 hectares in 2009, is currently at 428,000 ha (IBGE, 2009, 2020). This reduction is related to the drought that occurred between 2012 and 2016, which resulted in the death of plants, especially common cashew. On the other hand, even in the drought period, the area of dwarf cashew cultivation expanded in the main cashew producing regions. In the state of Ceará, there was an increase of 31,000 hectares in the harvested area from 2012 to 2017, indicating the resumption of cashew production (BRAINER; VIDAL, 2018) and the change in production system, with replacement of common cashew by dwarf cashew (SERRANO *et al.*, 2013).

In order to meet the resumption of cashew production, quality seedlings of clonal origin and grafted are required. Quality seedlings reduce the mortality rate of plants in the field, directly influencing the production capacity (SILVA *et al.*, 2019) and the uniformity of the orchard. For seedling quality, morphological parameters are used, but high-quality seedlings are produced at low cost, and have good survival, growth and development after planting in the field (MELO *et al.*, 2018).

In the production of seedlings, the choice of rootstock will influence the plant development and resistance to diseases and pests, as well as to environmental stresses (DONADIO *et al.*, 2019). Among the factors for seedling production, the substrate directly influences the initial formation, according to the structure, flow of water and air, in addition to nutrient concentrations to meet the demand of the seedlings.

The introduction of supplemented microorganisms in the substrate can bring benefits from the promotion of plant growth, improvement in plant nutrition, control of plant diseases, resistance to environmental stresses, and improvement in the biological quality of the soil (GARCIA; KNAAK; FIUZA, 2015; SILVA *et al.*, 2019). *Trichoderma* spp. are among the most studied microorganisms due to their various mechanisms of action in plant growth, development and yield (WOO *et al.*, 2014) and biosynthesis of phytohormones (auxins, gibberellins and cytokinins) (ANAM; REDDY; AHN, 2019). They also promote improvements in solubilization and supply of nutrients, such as phosphorus and micronutrients, mediated by the release of siderophores and secondary metabolites (TANDON *et al.*, 2018; ZHAO *et al.*, 2014). These microorganisms can produce organic chelating agents, responsible for increasing the availability and absorption of iron (LI *et al.*, 2018). Some strains under conditions of abiotic stress can improve photosynthetic efficiency and probably respiratory activity of plants, as a result of the reprogramming the expression of genes (SHORESH; HARMAN; MASTOURI, 2010).

Given the perspective of the potential for expansion of cashew production, the use of microorganisms such as *Trichoderma* species can be an alternative to supplement substrates, aiming to improve the availability of nutrients for rootstocks, and contributing to the production of more vigorous seedlings. Thus, the aim of this study was to evaluate the ability of *Trichoderma* strains (*T. longibrachiatum* and *T. asperellum*) to promote growth and improve nutrition in dwarf cashew rootstocks.

MATERIAL AND METHODS

Experimental location and design

The experiment was conducted in a shade net house with 50% of shading located in the Experimental Field of Pacajus, belonging to Embrapa Agroindústria Tropical, in Pacajus/CE, Brazil, with geographic coordinates: 4°11'12" S, 38°30'01" W and 79 m altitude. Environmental parameters were recorded throughout the experiment (April to June 2019) using a digital thermohygrometer: maximum and minimum daily temperatures (°C) and relative humidity (%) of 31.3 ± 1.5; 22.9 ± 1.0 and 83.9 ± 4.01, respectively.

The experimental design used was completely randomized, with three treatments (T1: control, T2: treatment with commercial inoculum *Trichoderma asperellum* and T3: treatment with inoculum 'LPPC299' *Trichoderma longibrachiatum*), with ten replicates. Each experimental unit consisted of one tube containing one seedling of dwarf cashew.

Production of inocula, quantification, applied rate and experiment installation

The strain *Trichoderma asperellum* was obtained from a commercial product, while the strain 'LPPC299' *Trichoderma longibrachiatum* is part of the work collection of the Postharvest Pathology Laboratory of Embrapa Agroindústria Tropical, collected in the rhizosphere of banana trees cv. Pacovan, in the city of Pacoti/CE.

To obtain the suspension and concentration of the commercial strain, 1 g of the product was weighed on an analytical scale and diluted in 9 mL of sterilized distilled water. Immediately after, the suspension was filtered with gauze in a beaker. The conidia were counted in a Neubauer chamber, which was filled with the suspension and placed under a light microscope (40x), obtaining a concentration of 5.4 x 10^9 conidia mL^{-1} (ALFENAS; MAFIA, 2016).

The strain 'LPPC299' was reactivated in Petri dishes containing PDA (potato, dextrose and agar) culture medium and incubated for seven days at 28 ºC with a photoperiod of 12 hours (light/dark) (ALFENAS; MAFIA, 2016). The inoculum was prepared from these colonies, by removing three discs (5 mm each) of PDA medium containing mycelium and conidia of the strain. These were subcultured to Erlenmeyer flasks containing 50 g of parboiled rice + 30 mL of deionized water (sterilized in autoclave at 121 ºC for 15 minutes). The inoculum was kept in BOD-type incubator at 28 °C with photoperiod of 12 hours (light/dark), for 15 days. After this period, the rice colonized by the fungus was aseptically transferred to sterile kraft paper envelopes and dried in BOD-type oven at a temperature of 32 °C for five days and then crushed in a blender. Conidia concentration of 3.2 x 10^9 conidia mL^{-1} was estimated according to the procedure described for the commercial strain.

Before installing the experiment, the tubes were subjected to disinfestation. For this, they were washed with neutral detergent and rinsed in running water and then submerged in 3% sodium hypochlorite solution, remaining for a period of 8 consecutive hours, being washed again in running water to remove excess hypochlorite. It was used commercial substrate (mixture of composted pine bark, peat and vermiculite) and had the following characteristics: bulk density = 427.7 kg m^{-3}; pH H_2O = 5.1; electrical conductivity = 0.9 dS m^{-1}; organic carbon = 226.6 g kg^{-1}; total-N = 7.9 g kg^{-1}; Ca = 309 mg L^{-1}; Mg = 251 mg L^{-1}; K = 375 mg L^{-1}; Na = 82 mg L^{-1}; P = 124 mg L^{-1}; Cl = 709 mg L^{-1}; NO_3^--N = 225 mg L^{-1}; NH_4^+-N = 2.1 mg L^{-1}, and SO_4^{2-}-S = 125.5 mg L^{-1}). The substrate was accommodated in cloth bags and sterilized in autoclave at 121 ºC for 1 hour; after 24 hours, the sterilization process was repeated, and then it was kept in a cold chamber until its use.

Tubes with capacity of 288 cm^3 were filled with the sterilized substrate, and one seed-nut of the genotype 'CCP 06' was sown in the center of each tube, 2 cm deep in the substrate, in the vertical position with the tip down. The 'CCP 06' is the most used genotype for rootstock production, as its seeds have high germination rate and are highly compatible with cashew genotypes used as scion (SERRANO *et al*., 2013). The plants were watered manually daily, according to the practices used by the cashew seedlings producers.

Trichoderma strains were applied in the form of suspension. The selected rate (based on the recommendation of the commercial product: 200 g of the product for every 100 kg of seeds, at the concentration of 10^9 spores mL^{-1}) was diluted in distilled and sterilized water, and 10 mL of the suspension were applied around the seed-nuts, according to the treatments. Irrigation was performed 24 hours after inoculation of the strains.

Evaluations of growth and nutritional status of the rootstock

At 60 days after germination, the seedlings were evaluated for the following growth-related variables: a) height, in cm (H, from the collar to the apical bud, measured with a measuring tape); b) stem diameter, in mm (SD, 5 cm from the substrate, determined with a digital caliper); c) number of leaves (determined by counting all fully expanded leaves of the plant); and d) leaf area, in cm^2 (measured with a leaf area integrator LI-3100C, LI-COR®), and Dickson's quality index obtained by the following formula DQI={(TDM)/[(H/SD)+(SDM/RDM)]}.

The seedlings were then separated into leaves, stem and roots, and washed with running water, 3% hydrochloric acid (HCl) (v:v) for 30 seconds and rinsed with deionized water. Subsequently, the different organs were placed in kraft paper bags and put in a forced air circulation oven at 65 ºC until reaching constant weight. After drying, the values of leaf (LDM), stem (STDM), root (RDM), shoot (SDM) and total dry matter (TDM), in g, were obtained.

The leaves were ground in a Wiley® mill and subjected to sulfuric digestion, followed by distillation and titration to determine total nitrogen contents and to nitric-perchloric digestion to determine phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese and zinc contents, by inductively coupled plasma optical emission spectrometry (ICP-OES). To determine boron content, the samples were incinerated in a muffle furnace, followed by quantification by spectrophotometry using the azomethine-H method (MIYAZAWA *et al*., 2009). Subsequently, the results of dry matter and nutrient contents in the leaves were used to calculate nutrient accumulations in the leaves.

Re-isolation of the fungal strains from plant tissue

The re-isolation of fungal strains was performed by the indirect root-plating method. Fragments with about 3 centimeters of root were washed with neutral detergent, dipped in a beaker containing distilled water, and then dried on sterile filter paper (ALFENAS; MAFIA, 2016). Subsequently, the fragments were deposited in Petri dishes containing PDA culture medium, supplemented with Rose Bengal (0.05 g L^{-1}) and incubated for 7 days at 28 ºC, with a photoperiod of 12 hours (light/dark). For the purification of

the strains, after 7 days of growth, a disc containing mycelium and conidia was plated in PDA medium and incubated under the same conditions as described above. These purified strains were those used in all trials of the present study.

Detection of growth promotion-related mechanisms in the fungal strains

a) In vitro production of 3-indoleacetic acid

To determine the qualitative and quantitative production of 3-indoleacetic acid (IAA) by *Trichoderma* strains, three discs of PDA culture medium (5 mm each), containing mycelium and conidia, were transferred to 250-mL Erlenmeyer flasks, containing 50 mL of PD (potato-dextrose) broth (sterilized at 121 ºC for 15 minutes), in the absence and presence of its precursor L-tryptophan. 5 mL of L-Tryptophan solution (100 μg mL^{-1}) were used for every 50 mL of liquid medium.

After seven days of incubation in an incubator shaker with orbital shaking of 150 rpm and temperature of 28 ºC, the colonized medium was centrifuged at 5,000 rpm for 15 minutes, collecting the supernatant. 3-indoleacetic acid was quantified using the colorimetric method, described by Gordon and Weber (1951), with 2 mL of the Salkowski reagent + 4 mL of the supernatant obtained from the isolate, which was kept in the dark at 28 ºC for 25 minutes to obtain the pink color. After the reaction time, the plant regulator was quantified in a spectrophotometer at 530 nm wavelength and the concentrations in μg mL^{-1} were calculated from the standard curve with known concentrations of the synthetic form of the plant regulator (0 to 100 μg mL^{-1}). With the readings, the concentration of IAA in the samples was calculated.

b) In vitro solubilization of calcium phosphate

The potential of *Trichoderma* strains in the solubilization of phosphates was verified by adding 100 mL of modified NBRIP culture medium (OLIVEIRA *et al*., 2012) in a 250-mL Erlenmeyer flask, with sterilization in autoclave at 121 ºC for 15 minutes. After cooling, three discs (5 mm each) containing PDA culture medium + mycelium and conidia were added to the medium. The material was incubated for seven days in an incubator shaker with orbital shaking of 150 rpm and temperature of 28 °C, and then centrifuged at 5,000 rpm for 15 minutes.

Phosphorus (P) content was quantified using 0.5 mL of the supernatant, 5.0 mL of distilled water and 2.5 mL of vanadate-molybdate solution. After 20 minutes of reaction protected from light, soluble phosphorus was quantified in a Cary 50 spectrophotometer at 420 nm wavelength. The concentration of solubilized P was determined using the colorimetric method of Murphy and Riley (1962), subtracting the P contained in the treatments by that contained in the control sample (NBRIP medium + phosphate and without inoculum). To calculate phosphorus concentrations in the samples, a standard curve of KH_2PO_4 was constructed (8 to 160 μg L^{-1}).

c) In vitro biosynthesis of siderophores

The production of siderophores by the strains was quantified by the universal method of Schwyn and Neilands (1987). In this method 100 mL of PD culture medium were added to a 250-mL Erlenmeyer flask and sterilized in autoclave at 121 ºC for 15 minutes. After cooling, three discs (5 mm each) of PDA medium containing mycelium and conidia were added to the medium. All Erlenmeyer flasks were incubated for a period of 7 days in an incubator shaker with orbital shaking of 150 rpm, at a temperature of 28 °C.

At the end of this period, all the material contained in the Erlenmeyer flasks was centrifuged at 5000 rpm to separate the fungal mass, and the supernatant was collected. A 2-mL aliquot of the supernatant was mixed with 2 mL of CAS (Chrome Azurol S) solution and mixed in glass tubes. The reaction mixture was maintained for 1 hour at room temperature and protected from light. Then, reading was performed in spectrophotometer, at 630 nm absorbance.

Statistical analysis

The data obtained were subjected to analysis variance by F test and, when significant, the means of the growth variables and nutrients were compared by the Scott-Knott test at 5% probability level. The means of microbiological analyses (production of 3-indoleacetic acid, phosphate solubilization and siderophore biosynthesis) were compared by the t test. The statistical analyzes were carried out through the AgroEstat program (BARBOSA; MALDONADO JUNIOR, 2015).

RESULTS AND DISCUSSION

Growth and nutritional status of the rootstock

The treatment 'LPPC299' - *Trichoderma longibrachiatum* influenced most of the biometric variables analyzed for the production of dwarf cashew rootstock (Table 1). The highest values were observed for plant height (27.81 cm), stem diameter (6.74 mm) and leaf area (423.90 cm^2). The commercial isolate *Trichoderma asperellum* did not differ from the control (without application of treatments). The increase in plant height and stem diameter promoted by the strain 'LPPC229' can be attributed in part to the ability of *Trichoderma* to regulate the respiratory system and metabolism of the plant, providing energy and sugar for growth (SHORESH; HARMAN; MASTOURI, 2010).

The increase in stem diameter in the rootstock phase, promoted by 'LPPC299', is desirable in the cashew seedling production system, since plants with larger diameter can be

grafted earlier, reducing the length of stay in the nursery (SERRANO; CAVALCANTI JUNIOR, 2016).

The absence of effect on the number of leaves of dwarf cashew can be justified by the non-production of 3-indoleacetic acid by the isolates (data not shown), since plant growth is directly correlated with the production of auxins, whose functions are linked to cell elongation, cell division, differentiation of vascular tissues, inhibition of leaf senescence and root growth (TAIZ *et al.*, 2017).

Santos *et al.* (2018) studied the growth of fruit seedlings under the action of plant growth-promoting microorganisms (*Trichoderma Bacillus* and *Azospirillum*) and verified that, for seedlings of common cashew (*A. occidentale*), the inocula did not influence the variables studied. Our results with dwarf cashew and *Trichoderma* differ from those found by authors. On the other hand, Sofo *et al*. (2011) analyzed the potential of *Trichoderma harzianum* in rootstocks of cherry (*Prunus cerasus* x *P. canescens*) and observed that plants inoculated with the strain showed a 61% increase in height.

Significant differences were also found in leaf, stem, shoot and total dry matter production, and the highest values were verified for the treatment 'LPPC299', compared to the other treatments. There was no significant difference between the Dickson Quality Index -DQI and the treatments (Table 2).

Similar results were found by López *et al.* (2019), who studied native strains of *Trichoderma* in the promotion of growth of yerba mate (*Ilex paraguariensis*) and verified that the inoculation of *Trichoderma atroviride*, *T. stibohypoxyli* and *T. koningiopsis* strains significantly increased shoot dry matter and total dry matter compared to non-inoculated plants.

Regarding the macro and micronutrient contents in cashew leaves, there was variation between the treatments for potassium, calcium and manganese, with lower values in the treatment 'LPPC299', resulting in reductions of 15.3, 20.9 and 21.0%, when compared to plants of the control treatment, while the commercial isolate did not differ from the control (Table 3).

Despite the variations in leaf contents of macro and micronutrients, the plants showed no symptoms of mineral deficiency. On the other hand, when considering the accumulation of macro and micronutrients in leaves of dwarf cashew rootstock (Table 4), no differences were

Table 1 - Development of 'CCP 06' dwarf cashew rootstock as a function of the application of *Trichoderma* strains

Treatments	Height cm	Diameter (mm)	Leaf area (cm^2)	Number of leaves
Control	24.75 b	6.32 b	347.70 b	9.80
Commercial	24.06 b	6.15 b	348.70 b	10.10
LPPC299	27.81 a	6.74 a	423.90 a	11.00
		F test		
Treatments	3.32*	6.25**	7.50**	1.41^{ns}
C.V. (%)	13.55	5.93	13.51	16.12

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level; (ns) Non-significant. ** and * Significant at 1% and 5% probability, respectively

Table 2 - Dry matter production of 'CCP 06' dwarf cashew rootstock as a function of the application of *Trichoderma* strains

Treatments	Dry matter					DQI
	Leaves	Stem	Roots	Shoot	Total	
	---------- g $plant^{-1}$ ----------					
Control	1.96 b	1.43 b	0.97	3.39 b	4.36 b	0.58
Commercial	1.96 b	1.55 b	1.01	3.51 b	4.52 b	0.61
LPPC299	2.35 a	1.86 a	1.11	4.21 a	5.32 a	0.67
			F test			
Treatments	2.93*	5.43*	1.15^{ns}	4.40*	3.76*	1.08^{ns}
C.V. (%)	19.90	18.67	20.62	18.03	17.71	20.63

Means followed by the same letter in the columns do not differ by Scott-Knott test at 5% probability level; $^{(ns)}$ Non-significant. ** and * Significant at 1% and 5% probability, respectively

verified between treatments, indicating that the variations in the contents were due to the dilution effect of these elements in the plant. This effect is characterized when the dry matter growth rate is higher than the absorption rate of the nutrient.

Studies have reported the direct or indirect influence of *Trichoderma* on nutrient contents in plants. Strains of the genus *Trichoderma* are able to solubilize nutrients in the soil, making them available to plants (FRANÇA *et al.*, 2017; TANDON *et al.*, 2018). However, these fungi can both solubilize and immobilize nutrients in their microbial biomass. In addition to the immobilization of nutrients in their microbial biomass, *Trichoderma* strains can also compete directly with plants for nutrients, when they are in extremely small amounts in the environment (LI *et al.*, 2018).

Table 3 - Macro and micronutrient contents in leaves of 'CCP 06' dwarf cashew as a function of the application of *Trichoderma* strains

Nutrients	Treatments			F test	C.V. (%)
	Control	Commercial	LPPC299		
	---------- g kg^{-1} ----------				
N	19.0	18.3	16.9	3.35^{ns}	7.16
P	1.4	1.3	1.3	3.70^{ns}	6.15
K	9.3 a	9.1 a	7.9 b	9.21**	6.53
Ca	6.5 a	6.3 a	5.2 b	8.24**	9.57
Mg	2.9	2.7	2.5	1.78^{ns}	14.42
S	1.1	1.0	0.9	3.70^{ns}	8.11
	---------- mg kg^{-1} ----------				
Cu	4	4	4	0.73^{ns}	14.89
Fe	39	37	33	1.04^{ns}	18.73
Zn	10	10	10	0.43^{ns}	9.60
Mn	279 a	273 a	220 b	5.52*	12.01
B	26	23	22	3.48^{ns}	8.70

Means followed by the same letter in the lines do not differ by Scott-Knott test at 5% probability level; $^{(ns)}$ Non-significant. ** and * Significant at 1% and 5% probability, respectively

Table 4 - Accumulation of macro and micronutrients in leaves of 'CCP 06' dwarf cashew rootstock as a function of the application of *Trichoderma* strains

Nutrients	Treatments			F test	C.V. (%)
	Control	Commercial	LPPC299		
	---------- mg $plant^{-1}$ ----------				
N	37.3	35.6	39.7	0.84^{ns}	13.60
P	2.8	2.5	3.0	2.12^{ns}	12.35
K	18.2	17.7	18.6	0.16^{ns}	14.05
Ca	12.7	12.5	12.1	0.09^{ns}	17.76
Mg	5.7	5.1	5.8	2.57^{ns}	9.98
S	2.1	2.0	2.1	0.58^{ns}	13.42
	---------- µg $plant^{-1}$ ----------				
Cu	8	8	9	0.24^{ns}	18.94
Fe	72	77	78	0.13^{ns}	24.53
Zn	20	20	23	1.60^{ns}	14.43
Mn	547	530	516	0.16^{ns}	16.39
B	50	43	55	3.40^{ns}	14.48

Means followed by the same letter in the lines do not differ by Scott-Knott test at 5% probability level; $^{(ns)}$ Non-significant. ** and * Significant at 1% and 5% probability, respectively

Detection of growth promotion-related mechanisms in the fungal strains

a) Production of 3-indoleacetic acid by the fungal strains

Regarding the production of 3-indoleacetic acid (IAA), *Trichoderma* strains were not able to produce IAA in vitro, both in the absence and in the presence of its precursor, L-tryptophan (data not shown). The results differ from those obtained by Ortuño *et al*. (2016), who found that all *Trichoderma* strains evaluated were able to produce auxins.

IAA production is dependent on the strain, and several external stimuli are associated with its production (NIETO-JACOBO *et al*., 2017). In vitro results do not always correspond to that observed in the soil, because the biosynthesis of secondary metabolites is dependent on environmental conditions. According to the same authors, the production of IAA does not seem to be a determining factor for the promotion of plant growth. According to Harman *et al.* (2004), differences between *Trichoderma* strains are expected and it is known that some strains are efficient in expanding the surface of the root system, enabling greater access to soil nutrients, while others are more efficient in solubilizing nutrients, producing auxins and/or biosynthesizing siderophores. In this context, this difference influences the capacity and potential of each isolate to perform its activities.

b) In vitro solubilization of calcium phosphate by the fungal strains

Trichoderma strains were able to solubilize calcium phosphate in vitro (Figure 1); however, no significant differences were observed between them. Species of the genus *Trichoderma* solubilize phosphates and micronutrients both in vitro and in the rhizosphere of plants (TANDON *et al*., 2018). This solubilization of phosphates mediated by *Trichoderma* is due to the exudation of low-molecular-weight organic acids, such as acetic, lactic, malic and oxalic acids, releasing phosphatase-type enzymes (BEHERA *et al*., 2014).

With solubilization, phosphorus concentrations in the soil increase, directly favoring the growth of the root system and resulting in the growth of the aerial part (SARAVANAKUMAR; ARASU; KATHIRESAN, 2013).

Evaluating the efficiency of the *Trichoderma asperellum* UFT 201 strain as a growth promoter in soybean, Chagas Junior *et al*. (2019) verified that the capacity of the strain to solubilize calcium phosphate was 67.8% higher than that of the control treatment (without strain), also resulting in a positive effect on the increase of biomass. In our study, the same positive effect could be seen, the *Trichoderma asperellum* strain was able to solubilize calcium phosphate and as a consequence it provided increases in leaf biomass, both aerial and total, compared to the control treatment.

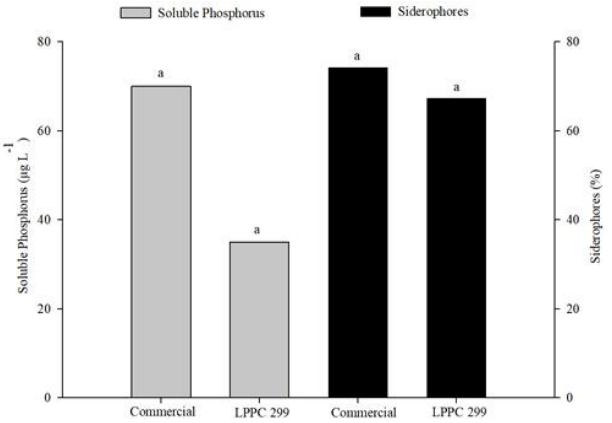
c) Biosynthesis of siderophores by the fungal strains

The fungal strains were also able to biosynthesize siderophores in vitro, in similar amounts (Figure 1). Siderophores exuded by fungi play an important role in the availability and absorption of iron by plants (MUKHERJEE *et al.*, 2018). Iron, in turn, performs functions in the plant related to the biosynthesis of chlorophyll, a pigment responsible for plant photosynthesis (TAIZ *et al.*, 2017).

Zhao *et al.* (2014) confirmed the ability of *Trichoderma asperellum* strains to mobilize insoluble Fe, making it available for plant growth. The authors indicated that the application of the strain in sterile soil increased the levels of Fe^{2+} and siderophores in the soil, as well as Fe^{2+} activity and Fe^{3+} chelate reductase activity in the roots of cucumber (*Cucumis sativus*).

Thus, due to the similarity between the strains in relation to the main growth-promotion mechanisms, it was not possible to explain the increase in the development of dwarf cashew rootstock seedlings promoted by *Trichoderma longibrachiatum* in comparison to the commercial product containing *T. asperellum*. The exudation of other phytohormones, such as cytokinins and gibberellins, not evaluated in the present study, may be related to the greater plant development. Cytokinins have effects on various physiological processes, of growth and development of plants. The gibberellin group is composed of hormones capable of promoting the stem extension and for inducing the mitotic division of leaf shoots (TAIZ *et al*., 2017).

Figure 1 - Contents of solubilized calcium phosphate (µg L^{-1}) and biosynthesis of siderophores (%) by the *Trichoderma* strains under study



Means followed by the same letter do not differ by t test at 5% probability level; Soluble phosphorus (F test.: 2.76^{ns}; C.V.: 69.45%;) Siderophores (F test: 1.38^{ns}; C.V.: 7.15%)

CONCLUSIONS

The strain 'LPPC299' - *Trichoderma longibrachiatum* promoted an increase in the growth of the 'CCP 06' dwarf cashew rootstock, so its use as a supplement for the planting substrate is recommended.

ACKNOWLEDGMENTS

The authors thank the Ceará Foundation for Support to Scientific and Technological Development (FUNCAP) for granting the scholarship to the first author under the process BMD-0008-01029.01.08/18, the Coordination for the Improvement of Higher Education Personnel (CAPES) for funding the research through PROAP resources (23067.037245/2019-31), and Embrapa Agroindústria Tropical for the infrastructure provided. The authors thank to Natália Moura de Vasconcelos Beleza post-harvest laboratory technician for her contribution to the analysis. The authors thank the Plataform of Sequencing LABCEN/CCB in the UFPE for the use of its facilities.

REFERENCES

ALFENAS, A. C.; MAFIA, R. G. **Métodos em Fitopatologia**. 2. ed. Viçosa: Universidade Federal de Viçosa, 2016. 516 p.

ANAM, G. B.; REDDY, M. S.; AHN, Y. H. Characterization of *Trichoderma asperellum* RM-28 for its sodic/saline-alkali tolerance and plant growth promoting activities to alleviate toxicity of red mud. **Science of the Total Environment**, v. 662, p. 462-469, 2019.

ASSOCIAÇÃO BRASILEIRA DE PRODUTORES EXPORTADORES DE FRUTAS E DERIVADOS. **Exportação de frutas cresce 16% em 2019**. 2019. Disponível em: https://abrafrutas.org/2020/01/28/exportacoes-de-frutas-cresce-de-16-em-2019/. Acesso em: 30 mar. 2020.

BARBOSA, J. C.; MALDONADO JUNIOR, W. **Experimentação agronômica e AgroEstat**: sistema para análises estatísticas de estatísticas de ensaios agronômicos. Jaboticabal: Multipress, 2015.

BEHERA, B. C. *et al*. Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove: a review. **Biocatalysis and Agricultural Biotechnology**, v. 3, n. 2, p. 97-110, 2014.

BRAINER, M. S. C. P.; VIDAL, M. F. Cajucultura nordestina em recuperação. **Caderno Setorial ETENE**, n. 54, p. 1-13, 2018.

CHAGAS JUNIOR, A. F. *et al.* Efficiency of *Trichoderma asperellum* UFT 201 as plant growth promoter in soybean. **African Journal of Agricultural Research**, v. 31, n. 5, p. 263-271, 2019.

DONADIO, L. C. *et al*. Dwarfing-canopy and rootstock cultivars for fruit trees. **Revista Brasileira de Fruticultura**, v. 41, n. 3, p. 1-12, 2019.

FRANÇA, D. V. C. *et al*. *Trichoderma* spp. isolates with potential of phosphate solubilization and growth promotion in cherry tomato. **Pesquisa Agropecuária Tropical**, v. 47, n. 4, p. 360-368, 2017.

GARCIA, T. V.; KNAAK, N.; FIUZA, L. M. Bactérias endofíticas como agentes de controle biológico na orizicultura. **Agricultural Microbiology**, v. 82, p. 1-9, 2015.

GORDON, S. A.; WEBER, R. P. Colorimetric estimation of indole acetic acid. **Plant Physiology**, v. 26, n. 4, p. 192-195, 1951.

HARMAN, G. E. *et al. Trichoderma* species-opportunistic, avirulent plant symbionts. **Nature Review Microbiology**, v. 2, n. 1, p. 43-56, 2004.

IBGE. **Levantamento sistemático da produção agrícola**: **Tabela 1** - Área, produção e rendimento médio - Confronto das estimativas Janeiro/Fevereiro - Brasil. Maio 2020. Disponível em: https://www.ibge.gov.br/estatisticas/ economicas/agricultura-e-pecuaria/9201-levantamento-sistematico-da-producao- agricola.html? edicao=27808&t=resultados. Acesso em: 17 set. 2020.

IBGE. **Produção agrícola municipal**: culturas temporárias e permanentes. Rio de Janeiro, 2009. v. 36, p. 1-93.

LI, M. *et al.* Effects of microbial bioeffectors and P amendements on P forms in a maize cropped soil as evaluated by ^{31}P–NMR spectroscopy. **Plant and Soil**, v. 427, n. 1, p. 87-104, 2018.

LI, Y. T. *et al*. Effects of *Trichoderma asperellum* on nutrient uptake and *Fusarium* wilt of tomato. **Crop Protection**, v. 110, p. 275-282, 2018.

LÓPEZ, A. C. *et al*. *Trichoderma* spp. from Misiones, Argentina: effective fungi to promote plant growth of the regional crop *Ilex paraguariensis* St. Hil. **Mycology**, v. 10, n. 4, p. 210-221, 2019.

MELO, L. A. *et al.* Qualidade e crescimento inicial de mudas de *Mimosa caesalpiniifolia Benth*. produzidas em diferentes volumes de recipientes. **Ciência Florestal**, v. 28, n. 1, p. 47-55, 2018.

MIYAZAWA, M. *et al*. Análise química de tecido vegetal. *In*: SILVA, F. C. **Manual de análises químicas de solos, plantas e fertilizantes**. 2. ed. Brasília: Embrapa Informação Tecnológica, 2009. cap. 2, p. 191-233.

MUKHERJEE, P. K. *et al*. Ferricrocin, the intracellular siderophore of *Trichoderma virens*, is involved in growth, conidiation, gliotoxin biosynthesis and induction of systemic resistance in maize. **Biochemical and Biophysical Research Communications**, v. 505, n. 2, p. 605-611, 2018.

MURPHY, J.; RILEY, J. P. A modified single solution method for determination of phosphate in natural waters. **Analytical Chemistry Acta**, v. 27, p. 31-36, 1962.

NIETO-JACOBO, M. F. *et al.* Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. **Frontiers in Plant Science**, v. 8, n. 102, p. 1-18, 2017.

OLIVEIRA, A. G. *et al.* Potencial de solubilização de fosfato e produção de AIA por *Trichoderma* spp. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 7, n. 3, p. 149-155, 2012.

ORTUÑO, N. *et al*. The use of secondary metabolites extracted from *Trichoderma* for plant growth promotion in the Andean highlands. **Renewable Agriculture and Food Systems**, v. 32, n. 4, p. 366-375, 2016.

SANTOS, C. H. B. *et al*. Promoting fruit seedling growth by encapsulated microorganisms. **Revista Brasileira de Fruticultura**, v. 40, n. 3, p. e-179, 2018.

SARAVANAKUMAR, K.; ARASU, V.; KATHIRESAN, K. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of *Avicennia marina*. **Aquatic Botany**, v. 104, p. 101-105, 2013.

SCHWYN, B.; NEILANDS, J. B. Universal chemical assay for the detection and determination of siderophore. **Analytical Biochemistry**, v. 160, n. 1, p. 47-56, 1987.

SERRANO, L. A. L. *et al*. Portaenxertos para a produção de mudas de cajueiro. **Pesquisa Agropecuária Brasileira**, v. 48, n. 9, p. 1237-1245, 2013.

SERRANO, L. A. L.; CAVALCANTI JUNIOR, A. T. Produção de mudas de cajueiro. *In*: SERRANO, L. A. L. **Sistema de produção do caju**. 2. ed. Fortaleza: Embrapa Agroindústria Tropical, 2016. cap. 5, p. 43-54.

SHORESH, M.; HARMAN, G. E.; MASTOURI, F. Induced systemic resistance and plant responses to fungal biocontrol agents. **Annual Review of Phytopathology**, v. 48, p. 21-43, 2010.

SILVA, C. F. B. *et al*. Uso do *Trichoderma* na cultura da banana. *In:* MEYER, M. C.; MAZARO, S. M.; SILVA, J. C. ***Trichoderma***: uso na agricultura. Brasília: Embrapa, 2019. p. 433-444.

SILVA, E. M. *et al*. Produção de mudas de cajueiro anão-precoce em substratos de resíduos orgânico. **Revista Brasileira de Agropecuária Sustentável**, v. 9, n. 1, p. 90-96, 2019.

SOFO, A. *et al*. *Trichoderma harzianum* strain T-22 induces changes in phytohormone levels in cherry rootstocks (*Prunus cerasus* X P. *canescens*). **Plant Growth Regulation**, v. 65, n. 2, p. 421-425, 2011.

TAIZ, L. *et al*. **Fisiologia e desenvolvimento vegetal**. 6. ed. Porto Alegre : Artmed, 2017. 888p.

TANDON, A. *et al*. Effect of *Trichoderma koningiopsis* on chickpea rhizosphere activities under different fertilization regimes. **Open Journal of Soil Science**, v. 8, n. 7, p. 261-275, 2018.

WOO, S. L. *et al*. *Trichoderma* - based products and their widespread use in agriculture. **The Open Mycology Journal**, v. 8, p. 71-85, 2014.

ZHAO, L. *et al*. Involvement of *Trichoderma asperellum* strain T6 in regulating iron acquisition in plant. **Journal of Basic Microbiology**, v. 54, p. S115-S124, 2014.