PAB
Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Pomology/ Original Article

Growth promotion of pecan rootstock seedlings with *Trichoderma* spp.

Abstract – The objective of this work was to select *Trichoderma* spp. strains with potential to promote the growth of rootstock seedlings of the pecan Barton cultivar by improving their morphological parameters and quality index, as well as to biochemically characterize these strains in vitro. In vitro bioassays were carried out with 59 *Trichoderma* spp. strains to evaluate their effects on the germination percentage and root growth of a bioindicator plant. The pre-selected strains were inoculated onto pecan seeds with a spore suspension at a concentration of $1x10^9$ conidia per milliliter. The experimental design was completely randomized, with five replicates. The analyzed variables were plant height, stem diameter, number of leaves, shoot dry mass, root dry mass, total dry mass, and the Dickson quality index. For each strain, qualitative evaluations of calcium phosphate solubilization and of the production of siderophores, hydrocyanic acid, and indole-3-acetic acid were carried out in vitro. The production of hydrocyanic acid and indole-3-acetic acid was not observed. Strains CPACT275, CPACT809, CPACT838, CPACT852, and CPACT859 significantly enhanced the biomass and quality index of 'Barton' pecan rootstock seedlings. The growth promoted by *Trichoderma* spp. may be related to calcium phosphate solubilization by all strains and siderophore production by CPACT838 and CPACT852.

Index terms: *Carya illinoinensis*, biostimulant, siderophores, solubilization of phosphate.

Carla Thais Rodrigues Viera[1] ✉, Yasmin dos Santos Boeira[1], Glaucia de Figueiredo Nachtigal[2], Carlos Roberto Martins[2] and Ricardo Alexandre Valgas[2]

[1] Universidade Federal de Pelotas, Campus Capão do Leão, Campus Universitário, s/nº, CEP 96160-000 Capão do Leão, RS, Brazil. E-mail: rodriguescarla835@gmail.com, y.santos.min@gmail.com

[2] Embrapa Clima Temperado, BR-392, Km 78, 9º Distrito, Monte Bonito, Caixa Postal 403, CEP 96010-971 Pelotas, RS, Brazil. E-mail: glaucia.nachtigal@embrapa.br, carlos.r.martins@embrapa.br, ricardo.valgas@embrapa.br

✉ Corresponding author

Received
April 26, 2024

Accepted
September 05, 2024

How to cite
VIERA, C.T.R.; BOEIRA, Y. dos S.; NACHTIGAL, G. de F.; MARTINS, C.R.; VALGAS, R.A. Growth promotion of pecan rootstock seedlings with *Trichoderma* spp. **Pesquisa Agropecuária Brasileira**, v.59, e03745, 2024. DOI: https://doi.org/10.1590/S1678-3921.pab2024.v59.03745.

Promoção de crescimento de mudas de porta-enxerto de nogueira-pecã com *Trichoderma* spp.

Resumo – O objetivo deste trabalho foi selecionar linhagens de *Trichoderma* spp. com potencial de promover o crescimento de mudas de porta-enxerto da cultivar de nogueira-pecã Barton ao melhorar seus parâmetros morfológicos e índice de qualidade, bem como caracterizar bioquimicamente essas linhagens in vitro. Foram realizados bioensaios in vitro com 59 linhagens de *Trichoderma* spp., para avaliar seus efeitos sobre o percentual de germinação e o crescimento radicular de planta bioindicadora. As linhagens pré-selecionadas foram inoculadas às sementes de nogueira-pecã com suspensão de esporos a $1x10^9$ conídios por mililitro. O delineamento experimental foi inteiramente casualizado, com cinco repetições. As variáveis analisadas foram altura de planta, diâmetro do caule, número de folhas, massa seca de parte aérea, massa seca de raízes, massa seca total e índice de qualidade de Dickson. Para cada linhagem, foram realizadas avaliações qualitativas da solubilização de fosfato de cálcio e da produção de sideróforos, ácido cianídrico e ácido indol-3-acético in vitro. Não foi observada produção dos ácidos cianídrico e indol-3-acético. As linhagens CPACT275, CPACT809, CPACT838, CPACT852 e CPACT859 promoveram aumento significativo na biomassa e no índice de qualidade das mudas de porta-enxertos de nogueira-pecã 'Barton'. A promoção do crescimento por *Trichoderma* spp. pode estar relacionada à solubilização de fosfato de cálcio por todas as linhagens e à produção de sideróforos por CPACT838 e CPACT852.

Termos para indexação: *Carya illinoinensis*, bioestimulante, sideróforos, solubilização de fosfato.

Introduction

The cultivation of pecan [*Carya illinoinensis* (Wangenh.) K. Koch] trees has been increasing in various countries, driven by the growing market demand for their fruits and derivative products, such as oil and flour, which are rich in mono- and polyunsaturated fatty acids, vitamin E, minerals, polyphenols, and phytosterols, offering numerous health benefits, particularly in the prevention of diabetes and cardiovascular diseases (Atanasov et al., 2018). Additionally, pecan trees can also be used as timber, ornament, shade source, and material for substrates (Martins et al., 2024).

The Southern region of Brazil has favorable soil and climate conditions for pecan trees, with the state of Rio Grande do Sul covering the largest planted area in the country, totaling 70% of the national pecan production and yielding an estimated 5.17 thousand tons in 2023 (Rio Grande do Sul, 2023). Although there are no consolidated official data, it is estimated that there are currently between 8 and 12 thousand hectares of pecan trees cultivated in the Brazilian territory, with an expected expansion to 25 thousand hectares by 2030 (Martins et al., 2024).

For the successful establishment of commercial orchards, in addition to a proper planning and preparation of the planting area, it is crucial to obtain high-quality seedlings, which includes parameters such as uniformity in height, a vigorous visual appearance, absence of pests and diseases, and a balanced ratio between the aboveground portion and the root system (Poletto et al., 2016). However, pecan producers face obstacles that make the activity time-consuming and costly, such as the need to carry out procedures as seed scarification and stratification, as well as to keep seedlings in a nursery for 24 to 36 months in order to reach the planting point (Poletto et al., 2016). Another difficulty is that the root system of nut trees is composed of a dominant (pivoting) main root and few secondary roots, which are responsible for absorbing water and nutrients (Grauke et al., 2016). Therefore, practices that favor and/or enhance the development of secondary roots can be beneficial to accelerate seedling growth.

In this scenario, the search for growth-promoting microorganisms becomes a challenge to be overcome in order to reduce the time the plants are kept in the nursery, operational costs, and cost of the seedlings to the producer, in addition to providing more vigorous and uniform seedlings, with a positive impact on the survival and initial development of plants in the field. In the literature, the high capacity of *Trichoderma* spp. to contribute to the sustainable development of agroecosystems is highlighted. These fungi stand out due to their abilities to activate plant defense mechanisms and preempt pathogen attacks, as well as to assist plants in resisting abiotic stresses and in the recovery of soils contaminated with heavy metals (Cortés-Hernández et al., 2023). Fungi of the genus *Trichoderma* spp. have been used in vegetable, forage, forestry, and fruit species due to their mechanisms involved in plant growth promotion, such as the solubilization of phosphate and micronutrients and the production of secondary metabolites and hormones (Santos et al., 2021).

The objective of this work was to select *Trichoderma* spp. strains with potential to promote the growth of rootstock seedlings of the pecan Barton cultivar by improving their morphological parameters and quality index, as well as to biochemically characterize these strains in vitro.

Materials and Methods

For the study, 59 *Trichoderma* spp. strains, belonging to the collection of microorganisms of interest for biological pest control of Embrapa Clima Temperado, were evaluated. For this, in vitro bioassays were initially carried out using lettuce (*Lactuca sativa* L.) as a bioindicator of the deleterious effects of the strains with harmful potential to pecan, according to the methodology of Kremer (2013). The strains were grown in rice bran culture medium (50 g rice bran extract, 20 g dextrose, and 15 g agar up to final volume of 1,000 mL in distilled water) and incubated for seven days at 25°C and in the absence of light, before being transferred into a 1.0 cm long groove close to the edge of 9.0 cm wide Petri dishes containing the already solidified culture medium. At the end of the incubation period, 1.0% (v/v) agar/water, at 25°C, was poured over the fungal colonies for the lettuce seeds to be laid on after solidification.

The Grand Rapids TBR curly lettuce cultivar was used for the bioassay. Seeds without pesticide treatment were disinfected by being immersed in 70% (v/v) alcohol for 1 min and in 1.5% (v/v) sodium

hypochlorite for 2 min, and then were washed four times with sterile water to remove residues. The seeds were arranged in parallel on plates, totaling 20 seeds per plate. At the end of three days of incubation at 25°C and a 16-hour photoperiod, the percentage of seed germination and the length of the primary root of the seedlings were evaluated. The experimental design was completely randomized, with four replicates per treatment. Petri dishes without fungal growth were used as controls. Means were compared by the Scott-Knott test, at 5% probability.

Seven promising strains were identified in the previous bioassay and then tested to evaluate their potential to promote the growth and improve the quality of rootstock seedlings of the Barton pecan cultivar. For this, another bioassay was conducted from August 2020 to January 2021 in a plant nursery, located at the Cascata experimental station of Embrapa Clima Temperado, in the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil. The nursery was completely enclosed, covered with agricultural plastic film, and equipped with a cooling system, with temperature and relative humidity control between 25 and 30ºC and between 70 and 80%, respectively. Seeds of the Barton pecan cultivar, without pesticide treatment, were disinfected following the same procedure applied to the lettuce seeds. Then, the seeds were scarified with a grinder and stratified using a plastic box containing layers of moist sand, at 4ºC, for 90 days in a cold chamber, according to the methodology of Poletto et al. (2016).

For the mass propagation of the strains, Erlenmeyer flasks containing 100 g parboiled rice and 70 mL distilled water were sterilized in an autoclave for 20 min at 120ºC. After the rice cooled, five disks (5.0 mm wide) of active colonies were removed for later use. At the end of a seven-day incubation period, at 25ºC and in the absence of light, 100 mL sterile water were added to each Erlenmeyer flask to obtain a spore suspension. The suspension was incorporated at a dose of 2.0 mL at $1x10^9$ conidia per milliliter and a depth of 5.0 cm in pots containing 4.0 kg washed sand and two pecan seeds. Once the seedlings were established, thinning was carried out to one seedling per pot.

Irrigation was performed with a graduated cylinder to avoid flooding of the substrate and leaching of the applied treatments. Weekly evaluations of morphological parameters began when most seedlings had two pairs of leaves, which occurred two months after planting, and continued until the sixth month. Plant height (cm) was determined by measuring the plant from its base to the insertion region of the youngest leaves, while stem diameter (mm) was measured at 3.0 cm from the base of the plant using a digital caliper. Only leaves larger than 2.0 cm were considered to calculate the mean number of leaves per seedling. To obtain shoot, root, and total dry mass (g), the seedlings were collected six months after planting, oven dried, and weighed on an analytical balance. The Dickson quality index (DQI) was determined according to the following equation (Dickson et al., 1960): DQI = (TDM) / [(H/SD) + (SDM/RDM)] where TDM is total dry mass, H is plant height, SD is stem diameter, SDM is shoot dry mass, and RDM is root dry mass.

Non-inoculated seedlings were used as the control. The experimental design was completely randomized, with five replicates, and the experimental data were tested for normality and homogeneity of variances. Once the assumptions were met, the results were subjected to the analysis of variance, and means were compared by the Duncan test, at 5% probability. If the assumptions were not met, the data were subjected to the non-parametric Kruskall-Wallis test, at 5% probability.

The seven strains previously selected were also analyzed for the following biochemical features: solubilization of calcium phosphate, and the production of siderophores and plant regulators such as hydrocyanic acid and indole-3-acetic acid.

The adapted methodology of Chagas et al. (2016) was used to evaluate the solubilization capacity of calcium phosphate. In this bioassay, mycelium disks (5.0 mm in diameter) were transferred to modified NBRIP culture medium (Nautiyal, 1999) containing 10 g L^{-1} glucose, 5.0 g L^{-1} $MgCl_2.6H_2O$, 0.25 g L^{-1} $MgSO_4.7H_2O$, 0.20 g L^{-1} KCl, 0.1 g L^{-1} $(NH_4)_2SO4$, 10 g L^{-1} Ca_3HPO_4, and 10 g L^{-1} agar. The bromothymol blue indicator was added to the medium, whose pH was adjusted to 7.0. The dishes were incubated at 25ºC, without light, for five days. Then, solubilization was evaluated qualitatively by observing if the blue color of the culture medium changed to yellow/orange around the fungal colony. Each fungal strain represented one treatment, and the dishes without inoculation were

used as the control. The experimental design was completely randomized, with four replicates.

To detect the production of siderophores, the strains of *Trichoderma* spp. were also subjected to a bioassay using the universal method of Schwyn & Neilands (1987). For this, six mycelial disks, measuring 5.0 mm in diameter, were transferred to Erlenmeyer flasks containing 100 mL potato-dextrose medium. After five days of growth, at 25°C, under orbital agitation at 150 rpm, the fungal mass was precipitated by centrifugation at 1,500 rpm for 10 min. The evaluation was carried out qualitatively after 15 min of mixing the supernatant and chromeazurol S solution, at pH 5.6, in equal volumes. The strains whose color changed from blue to yellow/brown were considered to have produced siderophores. Each fungal strain consisted of one treatment, and a BD medium without inoculation was used as the control. The experimental design used was completely randomized, with four replicates.

The methodology described by Miller & Higgins (1970) was adapted to conduct the bioassay to determine the hydrocyanic acid production of the seven selected strains. A 5.0 mm mycelial disk from each of strain was individually transferred to Petri dishes containing potato-dextrose agar (PDA) medium plus 4.4 g glycine ($C_2H_5NO_2$) and 0.081 g ferric chloride hexahydrate ($FeCl_3.6H_2O$). Adjusted to the diameter of the Petri dish lids, sterilized filter paper disks were immersed in a solution of 5.0% picric acid (w/v) and 2.0% sodium carbonate (w/v). The evaluation was carried out qualitatively after incubation for 15 days at 25°C and in the absence of light, based on the change in color from yellow to dark yellow or brown, indicative of hydrocyanic acid production by the strains. A PDA medium with $C_2H_5NO_2$ and $FeCl_3.6H_2O$, but without inoculation was used as the control. The experimental design was completely randomized, with four replicates for each treatment.

The in vitro production of indole-3-acetic acid was analyzed using the methodology adapted by Chagas et al. (2017). Five disks (3.0 mm in diameter) were transferred to Falcon tubes containing 25 mL potato dextrose plus L-tryptophan at a concentration of 100 mg L^{-1}. To investigate the synthesis capacity of the strains in the absence of the precursor, the same procedure was carried out using a potato dextrose medium without L-tryptophan. The used tubes were placed on an orbital shaker at 150 rpm, at 25°C, for eight days. Afterwards, 2.0 mL of the culture from each replicate were subjected to centrifugation at 12,000 rpm for 15 min. In Elisa plates, one part of the Salkowski reagent [$FeCl_3$ 0.5 mol L^{-1} + $HClO_4$ (35%)] and two parts of the supernatant obtained from each strain were added in the absence and presence of L-tryptophan. The evaluation was carried out qualitatively by observing the change in color from yellow to pink, indicative of the production of indoleacetic acid. The experimental design was completely randomized, with four replicates per treatment.

Results and Discussion

Of the 59 *Trichoderma* spp. strains evaluated in the bioassay using lettuce plants as a bioindicator of deleterious effects, 12 prevented completely seed germination, 17 were phytotoxic and reduced germination, and 30 did not differ significantly from the control (Table 1). Regarding the length of the primary root of the seedlings (Table 1), 24 strains promoted higher increases than the control, especially CPACT109 (280.20%) and CPACT275 (254.28%), followed by CPACT809 (197.75%), CPACT859 (189.18%), CPACT852 (183.88%), CPACT838 (166.94%), and CPACT815 (158.98%), which were selected for evaluation. Nandini et al. (2021) observed that, although the genus *Trichoderma* includes over 200 species, currently, *Trichoderma asperellum*, *Trichoderma viride*, *Trichoderma virens*, and *Trichoderma harzianum* are highlighted as the most commonly used in studies on plant growth promotion and pathogen control.

Morphological attributes, including plant height, stem diameter, and shoot and root dry matter, are important to determine seed quality during nursery cultivation, directly impacting the initial establishment of plants in the field. The evaluation of combinations of two or more morphological attributes can be carried out using indices or relationships, as the Dickson quality index (Grossnickle & South, 2017).

For most of the analyzed variables, except for plant height, stem diameter, and number of leaves per seedling, inoculation with *Trichoderma* spp. provided the best results (Table 2). With regard to number of leaves, the obtained results are in line with those of Santos et al. (2021), who also did not observe any significant difference between dwarf cashew

(*Anacardium occidentale* L.) seedlings inoculated with *Trichoderma* spp. and the control. According to the same authors, this occurred because the studied strains did not produce indole-3-acetic acid, an auxin directly related to the growth of plants, particularly of their aerial parts, which is attributed to the promotion of cell elongation in the apical region, as also observed in the present work.

Considering the relative difference between treatments with and without *Trichoderma* spp., significant increases were observed depending on the used strain, namely: from 17.56 to 55.12% for shoot dry matter, 67.58 to 82.54% for root dry matter, and 50.41 to 67.05% for total dry matter (Table 3). The relationship between shoot and root dry matter was significantly higher for strains CPACT809, CPACT838, and CPACT859 in relation to the control treatment, with a certain homogeneity, varying from 0.41 to 0.46 g, which is an indicative of some similarity in the attributes related to the morphological quality of plants compared with different strains of *Trichoderma* spp. These values show that there was the emission of

Table 1. Seed germination percentage (SGP) after three days of incubation, at 25ºC and a 16-hour photoperiod, and primary root length of seedlings of the Grand Rapids TBR lettuce (*Lactuca sativa*) cultivar in the absence (control) and presence of *Trichoderma* spp. strains[(1)].

Trichoderma spp. strain	SGP (number of seeds per plate)	Primary root length (mm)	*Trichoderma* spp. strain	SGP (number of seeds per plate)	Primary root length (mm)
Control	86.87a	4.90e	CPACT517	70.00b	5.77d
CPACT102	72.50b	1.03g	CPACT611	0.00e	0.00g
CPACT109	97.50a	18.63a	CPACT649	0.00e	0.00g
CPACT111	0.00e	0.00g	CPACT805	96.25a	10.95c
CPACT123	73.75a	0.96g	CPACT806	81.25a	6.73d
CPACT133	0.00e	0.00g	CPACT808	62.50b	6.08d
CPACT164	82.50a	8.25c	CPACT809	90.00a	14.59b
CPACT165	80.00a	9.12c	CPACT810	63.75b	5.68d
CPACT193	0.00e	0.00g	CPACT815	87.50a	12.69b
CPACT201	68.75b	2.14f	CPACT834	0.00e	0.00g
CPACT205	62.50b	0.87g	CPACT836	90.00a	8.17c
CPACT208	81.25a	1.91f	CPACT838	98.75a	13.08b
CPACT231	90.00a	8.62c	CPACT840	93.75a	9.42c
CPACT275	93.75a	17.36a	CPACT847	92.50a	9.36c
CPACT276	51.25c	0.65g	CPACT852	83.75a	13.91b
CPACT301	91.25a	8.62c	CPACT854	67.50b	6.45d
CPACT311	67.50b	0.73g	CPACT859	96.25a	14.17b
CPACT315	77.50a	6.35d	CPACT860	80.00a	9.45c
CPACT321	81.25a	3.31e	CPACT862	27.50d	0.83g
CPACT325	0.00e	0.00g	CPACT1055	91.25a	4.55e
CPACT331	48.75c	3.88e	CPACT1058	93.75a	1.37f
CPACT341	0.00e	0.00g	CPACT1059	41.25c	0.76g
CPACT362	81.25a	2.96f	CPACT1061	86.25a	1.52f
CPACT415	65.00b	1.32f	CPACT1063	75.00a	2.10f
CPACT418	61.25b	0.88g	CPACT1066	0.00e	0.00g
CPACT438	47.50c	4.10e	CPACT1105	93.75a	5.56d
CPACT445	56.25c	1.76f	CPACT1106	0.00e	0.00g
CPACT458	0.00e	0.00g	CPACT1108	78.75a	6.25d
CPACT459	82.50a	1.24f	CPACT1109	78.75a	2.94f
CPACT460	0.00e	0.00g	CPACT1163	55.00c	1.97f
CV (%)	20.60	31.46		20.60	31.46

[(1)]Means followed by equal letters, in the column, do not differ by the Scott-Knott test, at 5% probability. CV, coefficient of variation.

secondary roots capable of ensuring a rapid growth after planting, a desirable characteristic given the increase in the uptake capacity of the roots and in the resistance to water stress under field conditions. The ability of *Trichoderma* spp. to stimulate root growth makes it advantageous as a rooting agent, providing an alternative for reducing the use of chemical fertilizers (Ji et al., 2020).

Table 2. Chi-square and p-value for plant height, stem diameter, and number of leaves of six-month-old rootstock seedlings of the Barton pecan (*Carya illinoinensis*) cultivar produced from seeds subjected to 90 days of stratification and inoculated with *Trichoderma* spp. strains under plant nursery conditions, with temperature between 25 and 30ºC and relative humidity between 70 and 80%, at the Cascata experimental station of Embrapa Clima Temperado, located in the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil.

Variable	DF[(1)]	Chi-square	p-value
Plant height (cm)	5	2.30581[ns]	0.80541
Stem diameter (mm)	5	1.32000[ns]	0.93286
Number of leaves	5	5.65597[ns]	0.34115

[(1)]Degrees of freedom. [ns]Nonsignificant by the Kruskal-Wallis test, at 5% probability.

Table 3. Shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), and Dickson quality index (DQI) obtained for six-month-old rootstock seedlings of the Barton pecan (*Carya illinoinensis*) cultivar produced from seeds subjected to 90 days of stratification and inoculated with *Trichoderma* spp. strains under plant nursery conditions, with temperature control between 25 and 30ºC and relative humidity between 70 and 80%, at the Cascata experimental station of Embrapa Clima Temperado, located in the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil[(1)].

Trichoderma spp. strain	SDM	RDM	TDM	DQI
	----------(Gram per plant)----------			
Control[(2)]	2.05bc	4.01b	6.07c	0.98c
CPACT109	1.98bc	4.96b	6.94bc	1.22abc
CPACT275	2.41abc	6.72a	9.13ab	1.65a
CPACT809	2.99a	7.13a	10.12a	1.68a
CPACT815	1.73c	4.47b	6.20c	1.06bc
CPACT838	3.18a	6.96a	10.14a	1.55ab
CPACT852	2.72ab	7.32a	10.04a	1.75a
CPACT859	2.87a	6.98a	9.85a	1.56ab
CV (%)	17.34	16.63	15.62	20.86

[(1)]Means followed by the equal lowercase letters, in the columns, do not differ by the Duncan test, at 5% probability. [(2)]Without inoculation. CV, coefficient of variation.

Some promising strains promoted a greater increase from 58.16 to 78.57% in the Dickson quality index of the pecan rootstocks, especially CPACT275, CPACT809, CPACT838, and CPACT852 (Table 3). This result shows the superior quality of inoculated seedlings as this index considers parameters such as seedling vigor and the balance of biomass distribution (Oliveira et al., 2018). Although there is no standard value in the literature for the Dickson quality index of pecan seedlings, higher values are an indicative of a higher seedling survival in the field.

In plants grown under suboptimal conditions for their development, whether due to limitations in light, water, nutrients, temperature, or salinity, *Trichoderma* spp. may act as a mechanism to overcome stressful situations, promoting plant growth. This is because the fungus is capable of producing both antioxidant enzymes that reduce oxidative damage and abscisic acid that regulates stomatal closure (Stewart & Hill, 2014). In the present study, the response observed in seedlings treated with *Trichoderma* spp. was even more evident because, in all treatments superior to the control, the standard for the Dickson quality index (higher than 1.55) was reached under cultivation conditions in sand, a substrate in which few nutrients are available to plants.

Plant growth promotion by the *Trichoderma* spp. fungus occurs through secondary metabolites released into the rhizosphere, including small peptides and other active metabolites capable of promoting root branching and the solubilization of the insoluble micronutrients present in the soil in order to modulate root architecture, which ensures a greater absorption and translocation of scarcely available minerals, increasing plant development (Junges et al., 2016). The ability of all seven strains to solubilize calcium phosphate (Figure 1) and the production of siderophores by strains CPACT838 and CPACT852 (Figure 2) confirm this aspect. López et al. (2019) observed increases in the shoot and total dry matter of yerba mate (*Ilex paraguariensis* A.St.-Hil.), attributing this gain to the production of siderophores and the solubilization of phosphates by *Trichoderma* spp.

Growth promotion by *Trichoderma* spp. also occurs through indirect mechanisms, including microorganism control through competition for nutrients, antibiosis,

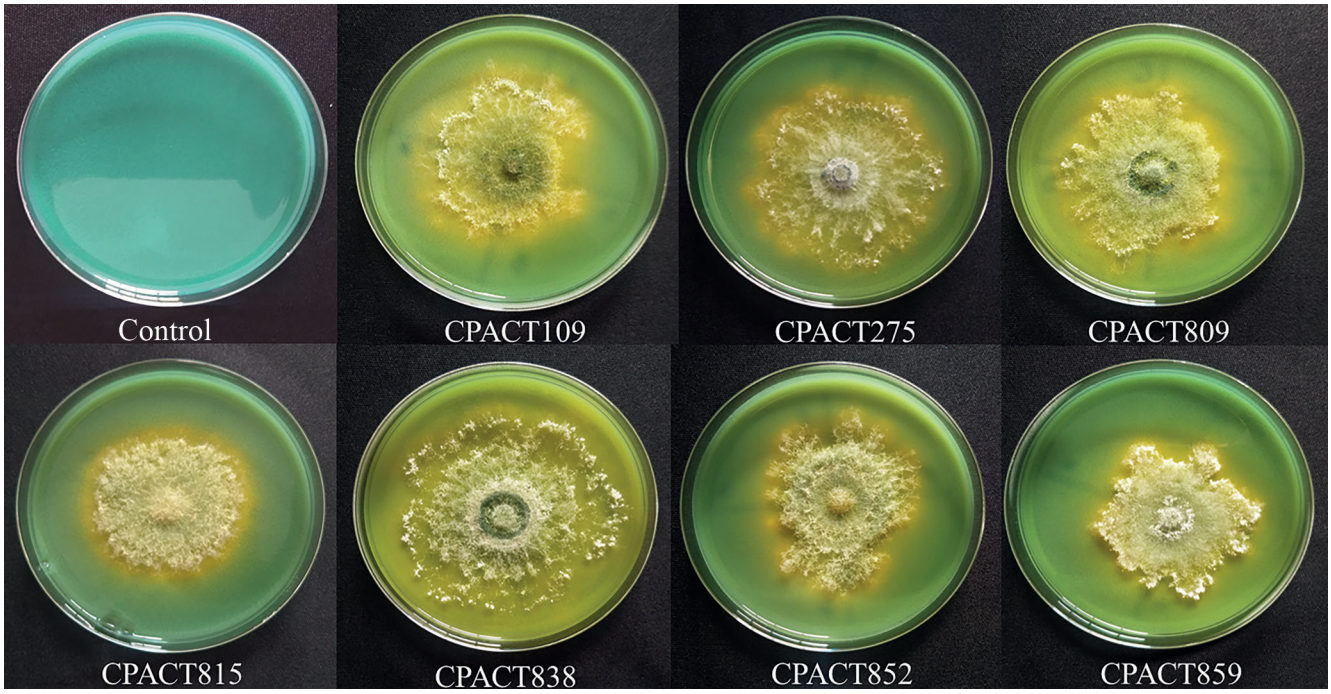

Figure 1. Qualitative evaluation of calcium phosphate solubilization by *Trichoderma* spp. strains in a modified NBRIP culture medium (control, without inoculation). The dishes were incubated, at 25ºC and without light, for five days. The change from the initial blue color to yellow/orange around the fungal colony is an indicative of solubilization, an indicator of the potential of the strains to promote the growth and increase the biomass and the quality index of pecan (*Carya illinoinensis*) rootstock seedlings.

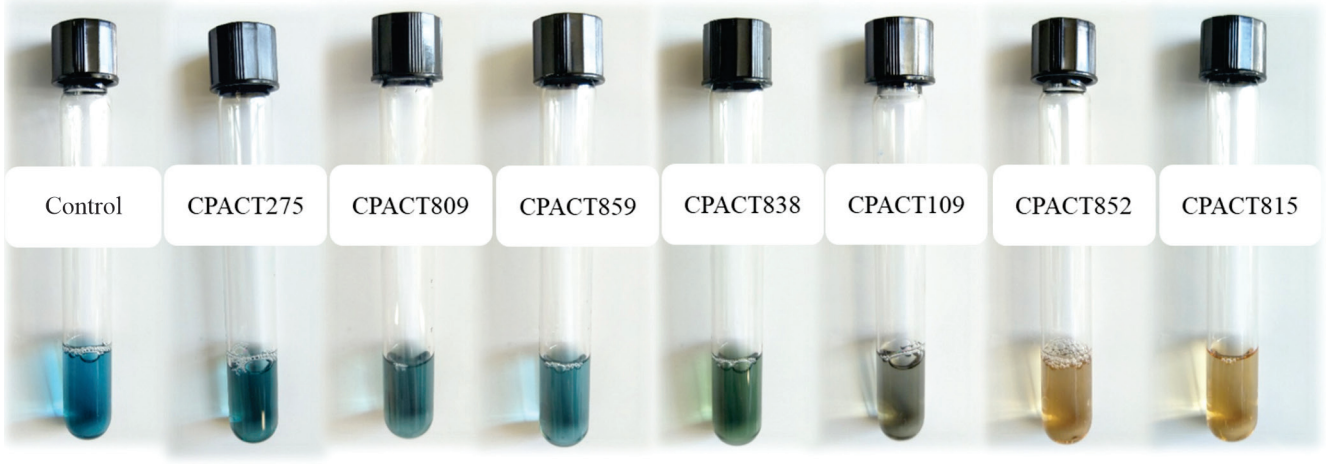

Figure 2. Qualitative evaluation of siderophore production by *Trichoderma* spp. strains in potato-dextrose medium (PD) for five days, at 25°C, under orbital agitation at 150 rpm. The change in the blue color of the solution to yellow/brown is indicative of siderophore production, an indicator of the potential of the strains to promote the growth and increase the biomass and the quality index of pecan (*Carya illinoinensis*) rootstock seedlings. A PD medium without inoculation was used as the control.

or mycoparasitism. In this process, extracellular lytic enzymes produced by *Trichoderma* spp. degrade the cell walls of pathogens, allowing the fungal hyphae to penetrate (Cortés-Hernández et al., 2023).

None of the seven strains of *Trichoderma* spp. tested showed the ability to produce hydrocyanic acid (Figure 3). Hydrogen cyanide can promote seed germination and act as a defense regulator in plants, inducing systemic resistance, although, at high concentrations, it can have deleterious and inhibitory effects (Díaz-Rueda et al., 2023).

The evaluated strains were also not able to produce indole-3-acetic acid, either in the presence or absence of the L-tryptophan precursor (Figure 4). In a similar bioassay, Santos et al. (2021) also found that strains of *Trichoderma* spp. used in dwarf cashew seedlings were not able to biosynthesize indoleacetic acid either in the absence or presence of L-tryptophan.

Different strains of *Trichoderma* spp. affect plants in various ways, producing over 800 known compounds (Cortés-Hernández et al., 2023). According to the same authors, some of these compounds, such as proteins and metabolites, activate immunity receptors in plants upon contact, aiding them in combating invasive organisms. Additionally, *Trichoderma* assists in soil recovery through heavy metal absorption and has the ability to survive in soil even in the presence of high concentrations of phytosanitary products. González-Pérez et al. (2018) added that one of the most accepted theories to explain the better shoot development in several plants during the interaction with *Trichoderma* spp. is the consequent production of volatile organic compounds, including mono- and sesquiterpenes, alcohols, ketones, and lactones, as well as the accumulation and redistribution of auxins in the roots in order to favor plant growth.

According to the obtained results, the selected *Trichoderma* spp. consist in a mechanism that promotes gains in the growth, development, and quality of the rootstock seedlings of the Barton pecan cultivar, proving to be an alternative of great technological innovation. In view of the observed gains, these strains

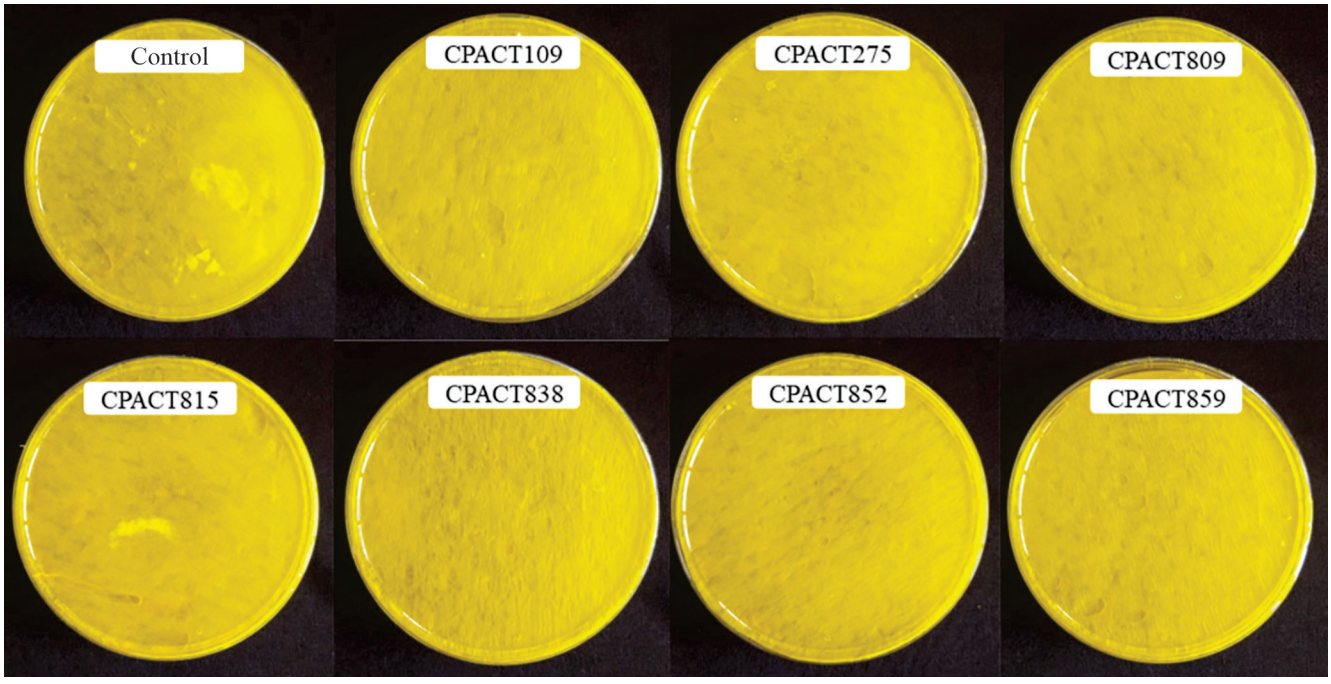

Figure 3. Qualitative evaluation of hydrocyanic acid production by *Trichoderma* spp. strains incubated for 15 days, at 25°C and in the absence of light, in dishes containing potato dextrose agar (PDA) medium plus 4.4 g glycine ($C_2H_5NO_2$) and 0.081 g ferric chloride hexahydrate ($FeCl_3$.6H2O). The change in the yellow color from medium to dark yellow or brown is an indicative of hydrocyanic acid production, an indicator of the potential of the strains to promote the growth and increase the biomass and the quality index of pecan (*Carya illinoinensis*) rootstock seedlings. Dishes without inoculation were used as the control.

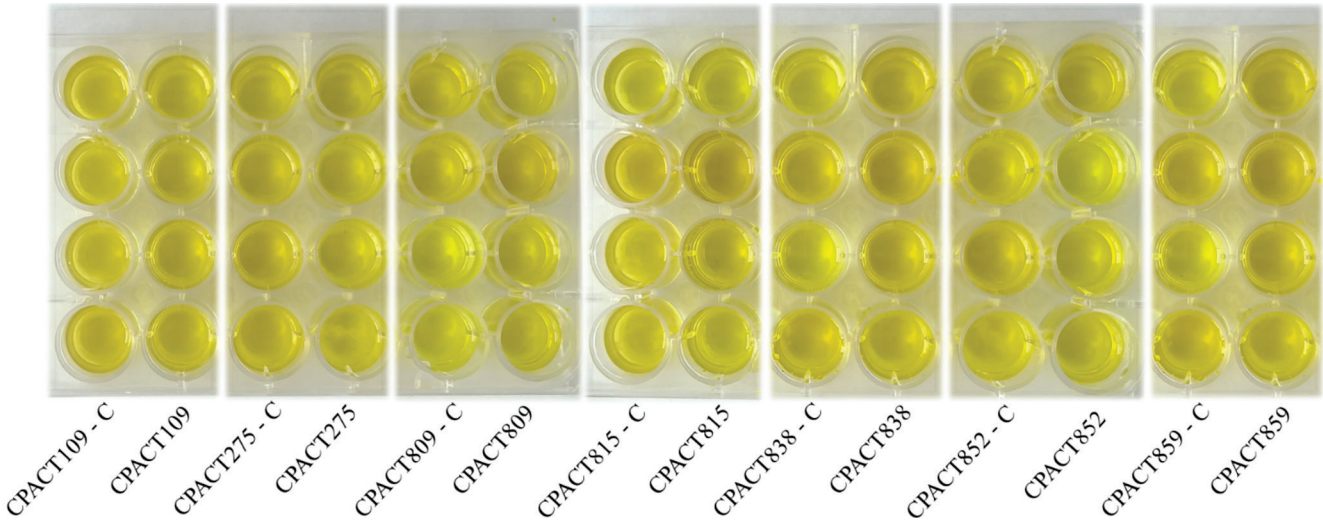

Figure 4. Qualitative evaluation of indoleacetic acid production by *Trichoderma* spp. strains in the absence (C) and presence of L-tryptophan in Falcon tubes containing potato dextrose medium, placed on an orbital shaker at 150 rpm and 25°C for eight days. The change in the yellow color of the Salkowski reagent to pink is an indicative of indoleacetic acid production, an indicator of the potential of the strains to promote the growth and increase the biomass and the quality index of pecan (*Carya illinoinensis*) rootstock seedlings.

of *Trichoderma* spp. showed the potential to increase seedling quality through growth promotion, which was not limited to the amount of active ingredient used, but related to inherent mechanisms of action.

Conclusion

The CPACT275, CPACT809, CPACT838, CPACT852, and CPACT859 *Trichoderma* spp. strains significantly enhance the biomass and quality index of the rootstock seedlings of the Barton pecan (*Carya illinoinensis*) cultivar, which may be related to calcium phosphate solubilization by all strains and siderophore production by CPACT838 and CPACT852.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for financial support; and to Universidade Federal de Pelotas (UFPEL) and to Embrapa Clima Temperado, for technical support.

References

ATANASOV, A.G.; SABHARANJAK, S.M.; ZENGIN, G.; MOLLICA, A.; SZOSTAK, A.; SIMIRGIOTIS, M.; HUMINIECKI, Ł.; HORBANCZUK, O.K.; NABAVI, S.M.; MOCAN, A. Pecan nuts: a review of reported bioactivities and health effects. **Trends in Food Science & Technology**, v.71, p.246-257, 2018. DOI: https://doi.org/10.1016/j.tifs.2017.10.019.

CHAGAS, L.F.B.; CASTRO, H.G. de; COLONIA, B.S.O.; CARVALHO FILHO, M.R. de; MILLER, L. de O.; CHAGAS, A.F.J. Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. **Brazillian Journal of Botany**, v.39, p.437-445, 2016. DOI: https://doi.org/10.1007/s40415-015-0247-6.

CHAGAS, L.F.B.; CHAGAS JUNIOR, A.F.; CASTRO, H.G. de. Phosphate solubilization capacity and indole acetic acid production by *Trichoderma* strains for biomass increase on basil and mint plants. **Brazilian Journal of Agriculture**, v.92, p.176-185, 2017. DOI: https://doi.org/10.37856/bja.v92i2.3221.

CORTÉS-HERNÁNDEZ, F. del C.; ALVARADO-CASTILLO, G.; SÁNCHEZ-VIVEROS, G. *Trichoderma* spp., una alternativa para la agricultura sostenible: una revisión. **Revista Colombiana de Biotecnología**, v.25, p.73-87, 2023. DOI: https://doi.org/10.15446/rev.colomb.biote.v25n2.111384.

DÍAZ-RUEDA, P.; RÍOS, L.M. de los; ROMERO, L.C.; GARCÍA, I. Old poisons, new signaling molecules: the case of hydrogen cyanide. **Journal of Experimental Botany**, v.74, p.6040-6051, 2023. DOI: https://doi.org/10.1093/JXB/ERAD317.

DICKSON, A.; LEAF, A.L.; HOSNER, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **The Forestry Chronicle**, v.36, p.10-13, 1960. DOI: https://doi.org/10.5558/TFC36010-1.

GONZÁLEZ-PÉREZ, E.; ORTEGA-AMARO, M.A.; SALAZAR-BADILLO, F.B.; BAUTISTA, E.; DOUTERLUNGNE, D.; JIMÉNEZ-BREMONT, J.F. The *Arabidopsis-Trichoderma* interaction reveals that the fungal growth medium is an important factor in plant growth induction. **Scientific Reports**, v.8, art.16427, 2018. DOI: https://doi.org/10.1038/s41598-018-34500-w.

GRAUKE, L.J.; WOOD, B.W.; HARRIS, M.K. Crop vulnerability: *Carya*. **HortScience**, v.51, p.653-663, 2016. DOI: https://doi.org/10.21273/HORTSCI.51.6.653.

GROSSNICKLE, S.C.; SOUTH, D.B. Seedling quality of southern pines: influence of plant attributes. **Tree Planters' Notes**, v.60, p.29-40, 2017.

JI, S.; LIU, Z.; LIU, B.; WANG, Y.; WANG, J. The effect of *Trichoderma* biofertilizer on the quality of flowering Chinese cabbage and the soil environment. **Scientia Horticulturae**, v.262, art.109069, 2020. DOI: https://doi.org/10.1016/j.scienta.2019.109069.

JUNGES, E.; MUNIZ, M.F.; MEZZOMO, R.; BASTOS, B.; MACHADO, R.T. *Trichoderma* spp. na produção de mudas de espécies florestais. **Floresta e Ambiente**, v.23, p.237-244, 2016. DOI: https://doi.org/10.1590/2179-8087.107614.

KREMER, J.R. Interactions between the plants and microorganisms. **Allelopathy Journal**, v.31, p.51-70, 2013.

LÓPEZ, A.C.; ALVARENGA, A.E.; ZAPATA, P.D.; LUNA, M.F.; VILLALBA, L.L. *Trichoderma* spp. from Misiones, Argentina: effective fungi to promote plant growth of the regional crop *Ilex paraguariensis* St. Hil. **Mycology**, v.10, p.210-221, 2019. DOI: https://doi.org/10.1080/21501203.2019.1606860.

MARTINS, C.R.; HAMANN, J.J.; ALBA, J.M.F.; FARIAS, R. de M. Pecã no Brasil. In: MARTINS, C.R.; LAZAROTTO, M.; MALGARIM, M.B. (Ed.). **Nogueira-pecã**: cultivo, benefícios e perspectivas. Brasília: Embrapa, 2024. p.37-52.

MILLER, R.L.; HIGGINS, V.J. Association of cyanide with infection of birdsfoot trefoil by *Stemphylium loti*. **Phytopathology**, v.60, p.104-101, 1970. DOI: https://doi.org/10.1094/phyto-60-104.

NANDINI, B.; GEETHA, N.; PRAKASH, H.S.; HARIPARSAD, P. Natural uptake of anti-oomycetes *Trichoderma* produced secondary metabolites from pearl millet seedlings – a new mechanism of biological control of downy mildew disease. **Biological Control**, v.156, art.104550, 2021. DOI: https://doi.org/10.1016/J.BIOCONTROL.2021.104550.

NAUTIYAL, C.S. An effect microbiological growth medium for screening phosphate solubilizing microorganisms. **FEMS Microbiology Letters**, v.170, p.265-270, 1999. DOI: https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.

OLIVEIRA, J.R. de; COSTA, C.A.S. da; BEZERRA, A.M.E.; ABUD, H.F.; LUCENA, E.M.P. de. Characterization of seeds, seedlings and initial growth of *Jacaranda mimosifolia* D. Don. (Bignoniaceae). **Revista Árvore**, v.42, e420403, 2018. DOI: https://doi.org/10.1590/1806-90882018000400003.

POLETTO, T.; MUNIZ, M.F.B.; POLETTO, I.; STEFENON, V.M.; MACIEL, C.G.; RABUSQUE, J.E. Dormancy overcome and seedling quality of pecan in nursery. **Ciência Rural**, v.46, p.1980-1985, 2016. DOI: https://doi.org/10.1590/0103-8478CR20150835.

RIO GRANDE DO SUL. Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação. **Nota técnica 32/23**: produção e mercado da noz-pecã 2023. Available at: <https://www.agricultura.rs.gov.br/upload/arquivos/202308/14143903-nt-32-noz-peca.pdf>. Accessed on: Aug. 10 2023.

SANTOS, J.M.R. dos; TANIGUCHI, C.A.K.; SILVA, C. de F.B. da; NATALE, W.; ARTUR, A.G. Trichoderma in the promotion of growth and nutrition of dwarf cashew rootstock. **Revista Ciência Agronômica**, v.52, e20207697, 2021. DOI: https://doi.org/10.5935/1806-6690.20210053.

SCHWYN, B.; NEILANDS, J.B. Universal chemical assay for the detection and determination of siderophores. **Analytical Biochemistry**, v.160, p.47-56, 1987. DOI: https://doi.org/10.1016/0003-2697(87)90612-9.

STEWART, A.; HILL, R. Applications of *Trichoderma* in plant growth promotion. In: GUPTA, V.K.; SCHMOLL, M.; HERRERA-ESTRELLA, A.; UPADHYAY, R.S.; DRUZHININA, I.; TUOHY, M.G. **Biotechnology and biology of *Trichoderma***. Boston: Elsevier USA, 2014. p.415-428. DOI: https://doi.org/10.1016/B978-0-444-59576-8.00031-X.