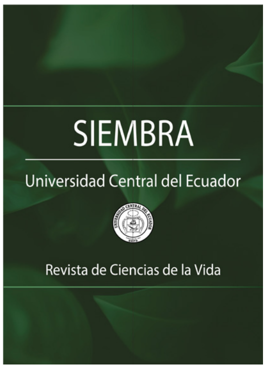
Original Article

Plant growth-promoting activity of four endophytic fungi isolated from Araucaria araucana

Actividad promotora del crecimiento vegetal de cuatro hongos endófitos aislados desde Araucaria araucana



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Abstract

Endophytic fungi [EF] are microorganisms that reside within plant tissues without causing apparent damage. They play a role in synthesizing growth-regulating substances, nitrogen fixation, protection against pathogens, and other benefits for plants. In this study, we evaluated the capacity of Indole Acetic Acid [IAA] production and phosphate solubilization in four strains of EF isolated from roots of *Araucaria Araucana,* and assessed their effect on the growth promotion of *Nicotiana tabacum*, as a model plant. Fungi were identified as *Phialocephala fortinii* (strain E-1), *Penicillium melinii* (strain E-13), *Umbelopsis dimorpha* (strain E-14), and *Preussia cymatomera* (strain E-36). The concentration of IAA was determined using the Salkowski method using potato dextrose broth [PDB] supplemented with 10 mg L^{-1} L-tryptophan, as a precursor for IAA synthesis. The yield was expressed in mg L^{-1}. The capacity of phosphate solubilization was determined in Pikovskaya solid medium through the evaluation of halos formed in the medium and calculating the relative solubilization efficiency ratio [RSE]. Finally, we evaluated the effect of EF in a co-culture with *N. tabacum* seedlings under *in vitro* conditions measuring aerial and root biomass of the seedlings. The highest values for IAA and RSE were observed in the case of *U. dimorpha* (52.29 and 3.36 mg L^{-1}, respectively). All fungi used promoted the growth of both aerial and root biomass of *N. tabacum* plants under *in vitro* conditions, obtaining the highest production of total dry biomass (aerial and root) with *U. dimorpha*, with a value of 188.5 mg, which was significantly higher ($p < 0,01$) than that of control plants (8.85 mg). Our findings suggest that EF isolated from *A. araucana* has the potential to promote plant growth and provide benefits to plants through the production of phytohormones.

Key words: plant growth, *Umbelopsis dimorpha*, IAA, phosphate solubilization.

Resumen

Los hongos endófitos [HE] son microorganismos que viven dentro de las plantas sin causar daño aparente, y colaboran en la síntesis de sustancias reguladoras del crecimiento, fijación de nitrógeno, protección contra

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patógenos, entre otros beneficios para las plantas. En este estudio se determinó la capacidad de producción de ácido indol acético [AIA] y de solubilización de fosfato, en cuatro cepas de HE aislados desde raíces de *Araucaria araucana* y se evaluó su efecto sobre la promoción del crecimiento en *Nicotiana tabacum*, utilizada como planta modelo. Los hongos fueron identificados como *Phialocephala fortinii* (cepa E-1)*, Penicillium melinii* (cepa E-13), *Umbelopsis dimorpha* (cepa E-14) y *Preussia cymatomera* (cepa E-36). El AIA fue determinado utilizando el método de Salkowski, usando caldo de papa dextrosa {CPD] suplementado con 10 mg L^{-1} de L-triptófano, como precursor para la síntesis de AIA, y la producción fue expresada en mg L^{-1}. La solubilización de fosfato fue determinada en medio sólido Pikovskaya, evaluando los halos formados en el medio y calculando el índice de eficiencia relativa de solubilización {ERS]. Finalmente, la estimulación del crecimiento vegetal por los HE en un co-cultivo con las plántulas de *N. tabacum,* en condiciones *in vitro,* fue evaluada a través de la producción de biomasa aérea y radicular de las plántulas. Los mayores valores de AIA y ERS fueron obtenidos con *U. dimorpha* (52,29 y 3,36 mg L^{-1}, respectivamente). Los hongos utilizados promovieron el crecimiento de la biomasa aérea y radicular de las plantas de *N. tabacum* en condiciones *in vitro*, obteniéndose la mayor producción de biomasa seca total (aérea y radicular) con *U. dimorpha*, con 188,5 mg, valor significativamente mayor ($p < 0{,}01$) que el de las plantas control (8,85 mg). A través de este estudio se infiere que los HE aislados de *A. araucana* promueven el crecimiento vegetal y brindan beneficios para las plantas mediante la producción de fitohormonas.

Palabras clave: crecimiento vegetal, *Umbelopsis dimorpha*, AIA, solubilización de fosfato.

1. Introduction

Plant growth-promoting fungi [PGPF] are microorganisms that reside in the rhizosphere of the plants and are known to improve the defense and growth mechanisms in plants (Parra Martínez, 2023). PGPF do diverse functions in plants, such as potential biocontrollers when competing for space and nutrients production of growth hormones, mineral solubilization, mycoparasitic and saprophytic resistance, root colonization and induced systemic resistance [ISR] in plants (Adedayo & Babalola, 2023). Endophyte fungi [EF] is one of the plant growth-promoting species.

EF is defined as microorganisms that spend most of their life cycle colonizing the host plant tissues without causing evident damage, and in some cases, they are even needed for the plant survival (Collinge et al., 2022; Ortega et al., 2020). This interaction between EF and its hosts is described as mutualistic (Card et al., 2016) because EF brings benefits to its host, which could exclude EF from pathogenic fungi and/or saprophytic fungi. Thus, microorganisms considered as endophytes (bacteria, fungi, viruses, protozoos) that at some point could cause harmful effects in the host plant, should be classified otherwise (Le Cocq et al., 2017). While the interaction fungus-plant is kept balanced, the fungus obtains nutrients from the host plant in exchange for benefits to the plant (Bamisile et al., 2018). Among these benefits, EF could give a higher content of nutrients, protection, radicular and aerial growth stimulation, and higher tolerance to stress (biotic and abiotic) when secondary metabolites are produced with a wide structural diversity that gives the plant protection and resistance against other herbivores, pathogen microorganisms and different environmental stressors (Sánchez-Fernández et al., 2013). These microorganisms have the capacity to synthetize plant hormones that control most of the physiological and biochemical processes, like cellular division, growth, aerial organ and plant root differentiation (Morocho & Leiva-Mora, 2019). One of the phytohormones secreted by some EF is the indole acetic acid [IAA], which is important in the processes related to plant growth, such as cellular elongation, development of vascular tissue and apical dominance (Andrade Ayala et al., 2020; Fu et al., 2015). Most of the mechanisms, growth regulations and plant development are produced thanks to phytohormones, being auxins, cytokines, gibberellins, abscisic acid, ethylene and salicylic acid the main ones. Auxins are a group of molecules found in plants, fungi and bacteria; the type of auxin that is more abundant in nature is IAA. Others, like indole propionic acid [4-CI-IAA] and indole-3-butyric acid [IBA], are found in fewer concentrations in plants (Báez-Pérez et al., 2015). Some studies done on IAA fungal producers, like in the genre *Trichoderma*, where 35 fungal strains were studied (*Trichoderma* sp.) due to their potential to synthesize this hormone (Ortuño et al., 2013).

Another relevant aspect of the EF capacity is the solubilization of nutrients, mechanism that can develop through the release of organic acids or by the secretion of enzymes (acid or basic phosphatases). This is fundamental for it is known that phosphorous [P] is a primary macronutrient for plant growth but a scarce one. It is thought that its use as commercial fertilizer is limited to the present century (Mogollón et al., 2018), being preponderant searching for alternatives for the solubilization of this nutrient. The conditions in which P is found in Chilean soils (associated to organic matter, precipitated with iron, calcium or aluminum compounds, immobilized or absorbed in clay particles) make necessary the search for ecological alternatives for this purpose. In this context, EF could play a fundamental role in the P cycle in soils because they have the ability to transform organic and inorganic phosphate when breaking the bonds that P has with metallic ions of iron, calcium, and aluminium, thus transforming it into soluble and available forms (Restrepo-Franco et al., 2015).

Araucaria araucana is an endemic species of the tempered forests of the Center-South and South zone of Chile and Argentinian Patagonia. It is characterized by its longevity and its cultural and scientific importance. In addition, it is a valuable economic resource for native people that live from the collection and use of the seeds as source of food and income. It is a unique genetic resource in the world (Hermann, 2006). The microbiome of *A. araucana* has not been studied much but there is an increase interest in studying the fungal species living in its surrounding. These species that come from external and changing environments, could hold a great biotechnological potential (Alarcón et al., 2020). In the study done by Chávez et al. (2023), different EF and arbuscular mycorrhiza were isolated from *A. araucana*, which were then inoculated in seedling of the same plant under hydric stress. The results showed a noticeable improvement in the resistance to hydric stress of the inoculated seedlings, as well as favorable changes in the morphology and physiology of the plants (Chávez et al., 2023). For this reason, it is interesting to evaluate if the interaction plant-fungus is beneficial for other species, specially in agricultural crops.

In this context, the hypothesis suggests that isolated EF from root of *Araucaria araucana* (millennial plant species) stimulates vegetal growth through the production of IAA and phosphate solubilization. The objective of this study was to prove the promoting capacity of the vegetal growth of four strains of EF through the production of phytohormones and solubilization of phosphate. The research was centered in determining the solubilization of phosphate in solid media and the concentration of produced IAA by EF in liquid fermentation. Additionally, growth stimulation in *Nicotiana tabacum* (model plant) in co-cultivation with EF was evaluated through the determination of the plant biomass. This data could serve as the base for future assays of optimization, where some cultivar conditions (media pH, cultivation time, tryptophan concentration) could be tested to increase the production of IAA.

2. Materials and Methods

2.1. Isolation and strain activation

EF used in this study were isolated from the roots of *A. araucana* (Chávez et al., 2023). The roots of the plants were carefully washed with water to eliminate soil remaining’s. Later, the roots were chopped in pieces of 0.5 cm approximately and were sterilized on the surface with ethanol at 70% for 1 min, sodium hypochlorite at 10% for 10 min. The sterilized roots were washed with sterile distilled water, dried and distributed in Petri dishes with malt extract agar [MEA] at 1% m/v. The samples were incubated at 24 °C for 7 days, and then the fungal strains were isolated and purified. The strains were molecularly identified as *Phialocephala fortinii* [strain E-1], *Penicilium melinii* [strain E-13], *Umbelopsis dimorpha* [strain E-14] y *Preussia cymatomera* [strain E-36], all of them belonging to the collection of strains of the Laboratory of Mycorrhizal Research and Biotechnological Applications of Fungi [LIMAB], University of Concepción, Los Angeles Campus. These strains were reactivated in MEA media at 1% m/v, placing a disk of agar/mycelium of 0.5 cm in the centre of the plates, and then incubated at 24 ± 1 °C under dark during a 10-day period.

2.2. Evaluation of indoleacetic acid (IAA)

The methodology described by Shahab et al. (2009) was used to determine the production of auxins from the different strains. The culture media of potato dextrose (PDB, Merck) was used complemented with L-tryptophan, 10 mg L^{-1}. Erlenmeyer flasks of 100 mL, containing 25 mL of the culture media, were inoculated with 2 disks of mycelium agar (5 mm) and then incubated for 7 days in a shaker at 120 rpm and 24 ± 1 °C. The flasks were inoculated in triplets per each fungal strain, and controls were prepared from the ones not inoculated. The liquid used for the determination of auxin concentration of type IAA was recovered through vacuum filtration (Shahab et al., 2009). For this purpose, the Salkowski reactant was prepared through a solution of 15 mL of $FeCl_3$ x $6H_2O$ (0.5 M) plus 00 mL of H_2SO_4 (98 % v/v) and 500 mL of distilled water (Glickmann & Dessaux, 1995). Afterwards, 1 mL of the supernatant was mixed with the Salkowski reactant and left at room temperature in dark conditions for 30 min. Later, the absorbance was measured at 530 nm in a spectrometer (TU-1810 Split Beam UV-VIS). A calibration curve was prepared to quantify the production of auxins from the known concentrations of IAA (10-50 mg L^{-1}).

2.3. Solubilization of solid phosphate culture medium

The solubilization of phosphate was evaluated in solid culture medium for the four species of EF mentioned in 2.1. The Pikovskaya culture medium [PVK] (Sanchez-Gonzalez et al., 2023) containing $(NH_4)_2SO_4$ (0.5 g), KCl (0.2 g), $MgSO_4 \times 7H_2O$ (0.1 g), $MnSO_4 \cdot H_2O$ (0.004 g), NaCl (0.2 g), D–Glucose (10 g), $FeSO_4 \times 7H_2O$ (0.002 g), $Ca_3(PO_4)_2$ (5g), yeast extract (0.5 g), chloramphenicol (0.1 g) and agar 1 % m/v was used. The media was sterilized at 121 °C for 15 min, and once warm, 25 mL of the media were placed in Petri dishes under the laminar flow chamber. After the solidification of the PVK medium, the Petri dishes were inoculated with 0.5 cm diameter disks of agar/mycelium. The plates were incubated at dark at 24 ± 1 °C and every 5 days the diameter of the colonies and the halo of solubilization around the colonies (mm) were measured, which shows the solubilization of the insoluble phosphate in the culture media. The index of relative efficiency of solubilization [RSE] was applied to estimate the solubilized phosphate, calculated by the equation [1] described by Romero-Fernandez et al. (2018).

$$RSE = \frac{a+b}{a} \qquad [1]$$

where:

- *a* =diameter of a colony (mm)
- *b* = diameter of the solubilization halo (mm)

The criteria stablished by Silva-Filho and Vidor (2000) was used to categorize the level of solubilization of the strains, which shows that the capacity of solubilization is considered low when the RSE is lower than 2, medium is higher than 2 but lower than 3, and high if it is higher than 3.

2.4. Endophyte Fungi as plant growth promoters

2.4.1. Plant material and growth conditions

Nicotiana tabacum seeds were used for the co-cultivation, along with the mentioned fungal strains in the part 2.1. The seeds were disinfected on the surface with ethanol at 70% treatment for 1 min, followed by sinking in 3% sodium hypochlorite and a drop of Tween 20 for 10 min, then shook in a vortex for 2 min. Finally, they were washed with sterile distilled water three times consecutively previous to the sowing. A complete basal medium Murashige & Skoog [MS], supplemented with 3% saccharose and 0.7% agar, adjusted to pH 5.6 and then sterilized for 15 min at 1 atm and 121 °C, was used for the seed germination (Vilariño-Rodríguez, 2022).

2.4.2. Co-cultivation of plants and fungi

After the germination of tobacco plants, they were transferred to a squared agar plate with MS media supplemented with 0.5% of saccharose (p/v), and at this moment, the fungi were used for the inoculation. Two disks of mycelium agar (5 mm of diameter) cut with a sterile tooth stick were placed at a distance of 1.5 cm in between and at 5 cm of distance from the radicle (3 seedlings per plate) according to the protocol from Dovana et al. (2015). The lower part of the plate was covered with aluminum foil to avoid the light from the camera to affect the mycelium, but part of the seedling was left exposed to the light. A photographic record was done every 5 days after the inoculation [dai] to evaluate plant growth. After 15 days, a destructive harvest was performed, where the foliar and radicle growth was evaluated to determine the dry weight (mg) of each plant.

2.5. Statistical Analysis

The normality of the data and variance homogeneity were evaluated by the tests Shapiro-Wilk and Levene, respectively. The statistics significance was determined using the Tukey Test ($p < 0.05$). The statistical analyses were done using the software Statistica and 10 (Statsoft). Each assay was performed three times ($n = 3$).

3. Results and Discussion

3.1. Concentration of indoleacetic acid [IAA]

The four strains had the capacity to produce IAA, and as it can be observed in Figure 1, the only strain with significant differences in the production of the hormone was *U. dimorpha* strain E-14 (52.29 mg L^{-1}), and for the strains E-1, E-13 and E-36 there were no significant differences (17.2, 9.2 and 21.1 mg L^{-1}, respectively). Six different biosynthetic pathways of IAA were identified, five of them depend on tryptophan (Kejela, 2024). Therefore, the results suggest that the production of IAA by the distinct fungi could have a synthesis dependent on this precursor.

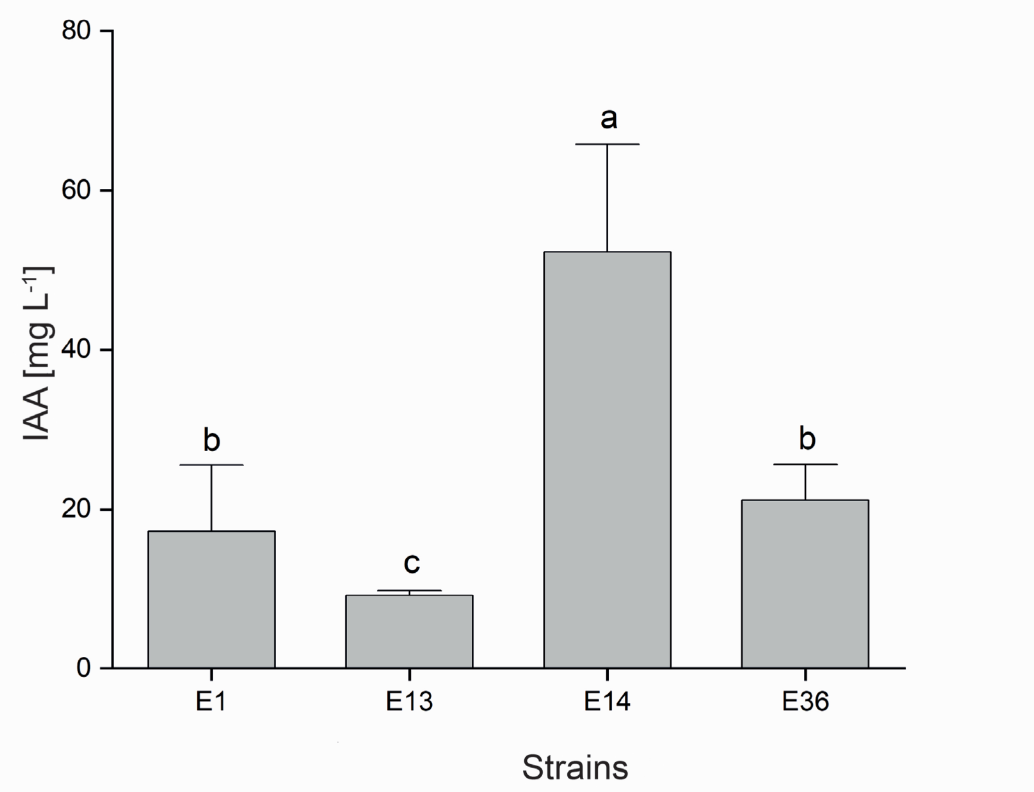

Figure 1. Determination of auxin production (IAA) for the different EF strains evaluated in PD medium. *Phialocephala fortinii* (E-1), *Penicillium melinii* (E-13), *Umbelopsis dimorpha* (E-14) and *Preussia cymatomera* (E-36). Data are expressed as mean ± standard deviation. Values followed by the same letter do not show significant differences ($p < 0.05$).

It was reported that *U. dimorpha* JSNL001-1 has shown a significant effect in the improvement of growth rate and drought resistance in *Dendrobium officinale* (Xú, 2014). This EF, in addition to the production of IAA as demonstrated in this study, has the capacity to secrete distinct phenolic compounds and organic acids that promote plant growth, such as described by Qin et al. (2018). They evaluated the production of diverse secreted metabolites by *U. dimorpha* in interaction with *Kadsura angustifolia*, changing some host metabolites. The increase of lateral roots can increase the synthesis or production of volatile compounds that activate genes related to enzymes of the biosynthetic pathway of auxins (Hermosa et al., 2012). The results showed that the fungi hold potential for the production of IAA, promoting plant growth of the lateral roots and aerial biomass (Figure 3). In *Aspergillus awamori*, it was found a production of 24.2 µg mL^{-1} of IAA (Mehmood et al., 2019), and for *Preussia* sp. BSL-10, cultivated in growth media Czapek for 14 days, 1.64 ± 0.15 µg ml^{-1} of IAA were detected (Al-Hosni et al., 2018). In addition, in other fungi (edible), such as in *Morchella importuna* species, a production of IAA close to 80 mg L^{-1} was reported (Arroyo Marín, 2023), higher than the quantity reported in this study.

Rhizosphere's microbiome favours plant growth by stablishing mutualistic interactions between plants and microorganisms, which secrete diverse compounds that promote the synthesis of beneficial metabolites in plants for its development (Pascale et al., 2020). However, microorganisms can independently synthetize metabolites that promote the growth of plants, such as phytohormones, siderophores and enzymes that facilitate the solubilization of phosphate (Kumar, 2016). Naureen et al. (2022) isolated 150 fungi from the rhizosphere and root endosphere of the perennial pastures *Cymopogon jwarancusa* and *Panicum antidotale.* They also evaluated the capacity of the fungi to stimulate plant growth of *Arabidopsis thaliana* (Col-0). The authors showed that EF strains CJAN 1179 and PAAN 1135 caused a significant stimulation of radicle growth with the formation of secondary roots just after 16 days of plant inoculation. Moreover, the strain CJAN 1179 produced 1.638 µg ml^{-1} of IAA in the presence of tryptophan as precursor (Naureen et al., 2022).

3.2. Solubilization of phosphate

The evaluation of solubilization activity produced a positive reaction with the formation of hyaline halos around the colonies (Figure 2b). The solubilization halo started to be observed around the third to fifth day in the PVK medium. The strain E-1 was the only one that did not present a solubilization halo during the assay (RSE = 1). According to Jones et al. (1991) and Whitelaw (1999), the absence of solubilization halos in the solid culture media did not necessarily indicate the lack of solubilization capacity in the organism. Therefore, it is necessary to use liquid media to obtain more accurate results. According to Mikheev et al. (2022), phosphate solubilization in *P. fortinii* is attributed to the production of acid phytase (maximum of 6.91 ± 0.17 U the 21st culture day in PDB) and to the capacity to accumulate polyphosphate in the hyphae cells. Thus, it cannot be discarded that under different culture conditions, this strain (E-1) could present solubilization activity.

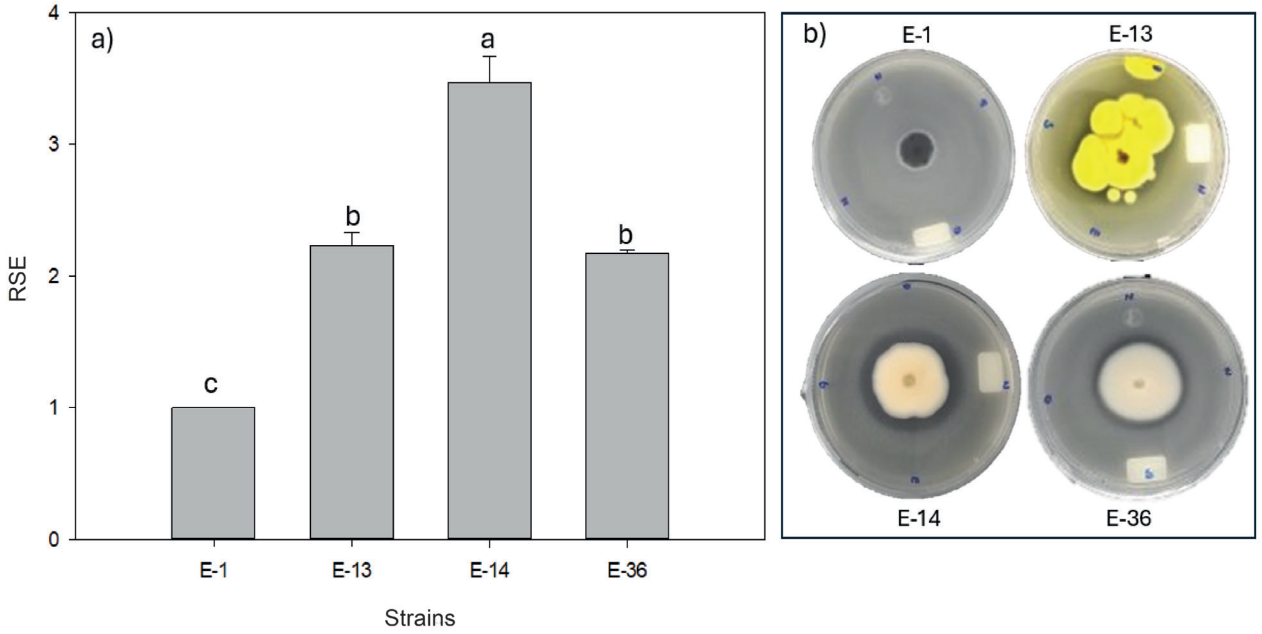

Figure 2. a) Relative solubilization efficiency ratio [RSE] at different growth periods (5, 10 and 15 days). Data are expressed as mean ± standard deviation. Values followed by the same letter do not show significant differences ($p < 0.05$). b) Solubilization halos (hyaline zone) of *Phialocephala fortinii* (E-1), *Penicillium melinii* (E-13), *Umbelopsis dimorpha* (E-14) and *Preussia cymatomera* (E-36), after 10 days of growth in PVK medium.

At 10 days of growing (Figure 2a), it was observed that E-14 was the strain with the highest index of RSE (3.36), being significantly higher to the solubilization indexes presented by the other strains. The development of the halo around the fungi colony can be explained by the secretion of enzymes and/or organic acids. The organic acids can acidify the cells of the surrounding environment of the fungi causing the release of P ions through H$^+$ substitution (joined to phosphate), thus, producing soluble phosphorous for plant use (Park et al., 2009; Saeed et al., 2021). On the other hand, organic acids can chelate the cation joint to the phosphate with their hydroxyl and carboxyl groups making P available in form of orthophosphate (Picone & Zamuner, 2002). During a study where phosphate solubilization fungi were isolated and characterized to inoculate coffee plants, 151 isolates were analyzed from which 21 presented the capacity to solubilize phosphate. Species of the genre *Umbelopsis* and *Penicillium* were found, but *Penicillium* species showed the highest solubilization capacity (Arias et al., 2023).

According to the RSE index, the values for the strains E-13 and E-36 (2.23 and 2.18, respectively) were considered medium and for the strain E-14 (3.36), high. The values in this study are higher than the values found by Morales et al. (2011), who reported a maximum solubilization index of 1.3 for *Penicillium albidum*, *P. thomii*, *P. restrictum*, *P. frequentans*, *Gliocladium roseum* and *Penicillium* sp. strains. However, the values obtained are less than the reported by Hajjam and Cherkaoui (2017), who reported solubilization index values of up to 5.3 for *Trichosporon beigelii*. Arias-Mota et al. (2022) reported RSE values in the range of 1.13 – 6.5 in rhizosphere fungi isolated from coffee plants, which shows the need to perform initial selection tests to obtain strains with biotechnological potential.

3.3. Plant-growth stimulation

The aerial biomass in *N. tabacum* plants inoculated with EF, showed a significant increase with respect to the control plants in all the studied strains. The strains E-1 and E-14 stood out as they stimulated the highest rates of growth but without significant differences between them (39.8 and 37 mg, respectively). The values represented an increase of 5.78 and 6.21 times with respect to the control plants. At the radicle level, the strains E-1, E-14 and E-36 showed significant differences in relation to the control, obtaining the highest dry weight with E-14 (72.5 ± 0.9 mg) (Figure 4). This could be related to most of the production of IAA produced by the strain E-14 (Figure 1). It is known that auxins are responsible of starting the formation of the lateral roots affecting the cellular cycle, activating founder cells present in the pericycle (Glick et al., 2012; Naureen et al., 2022). This phenomenon was observed in *N. tabacum* plants that developed secondary roots (Figure 3) from 10 dai, and completely different to the control plants that did not have secondary root development. In similar studies where the endophyte fungus *Cyanodermella asteris* was tested in *A. thaliana* plants, the roots presented a greater lateral growth, higher biomass, and shorter main root in answer to the auxin detected at the root ends and lateral primordia (Jahn et al., 2021).

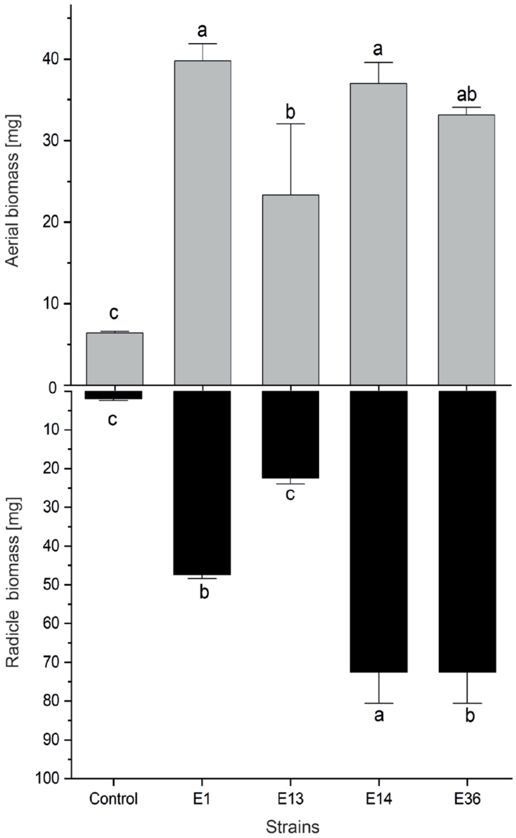

Figure 3. Growth of *N. tabacum* at 5, 10 and 15 days after inoculation with different strains of EF *Phialocephala fortinii* (E-1), *Penicillium melinii* (E-13), *Umbelopsis dimorpha* (E-14) and *Preussia cymatomera (*E-36).

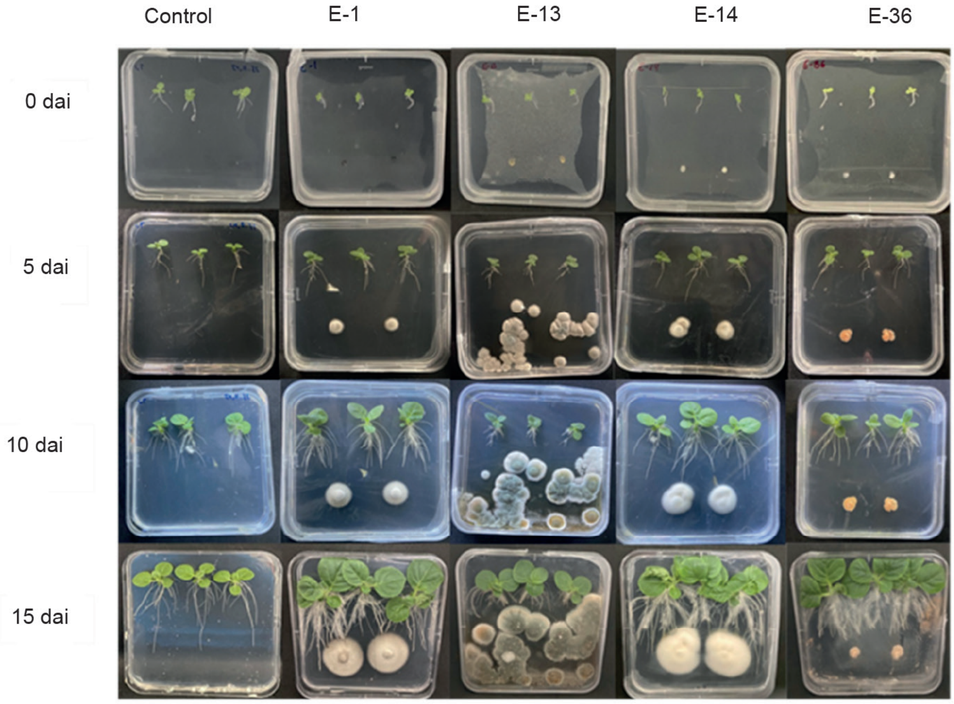

Figure 4. Aboveground and root biomass of *N. tabacum pl*ants inoculated with different strains of EF: *Phialocephala fortinii* (E-1), *Penicillium melinii* (E-13), *Umbelopsis dimorpha* (E-14) and *Preussia cymatomera* (E-36). Data are expressed as mean ± standard deviation. Values followed by the same letter do not show significant differences ($p < 0.05$).

The strain E-13 did not promote radicle development due to an abundant sporulation of the fungus. This inhibited the total plant growth (Figure 3) despite of the strain's capacity of IAA secretion (Figure 1). The production of phytohormones, like IAA, was observed in studies done on *P. citrinum* isolated from *Triticum aestivum* L. under drought-stress conditions (Kaur & Saxena, 2023). The authors proposed that tolerant-to-drought fungi, such as *P. citrinum,* activate the adaptive systems of wheat and, at the same time, grant protection and plant growth stimulation (Kaur & Saxena, 2023). In another study where the halotolerant fungus *P. olsonii* was evaluated in tobacco plants in hydroponic cultivation, an increase in tolerance to salinity was observed, stimulating plant growth and reducing the use of chemical fertilizers to half (Tarroum et al., 2022).

4. Conclusions

All the species/strains evaluated in this study produced phytohormones of the auxin type [IAA]. The species *Umbelopsis dimorpha* (strain E-14) presented the highest production of IAA. The EF had the capacity to solubilize phosphate except for *Phialocephala fortinii* (E-1), which was not able to solubilize phosphate in solid PVK medium. The EF isolated from *Araucaria araucana* roots promoted plant growth in *N. tabacum* by the production of IAA and phosphate solubilization. This opens the possibility of testing these fungi or fungal extractions in other agricultural/forest interesting crops.

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Contributor roles

- Carolina Díaz-Valenzuela: conceptualization, investigation, methodology, software, formal analysis, writing – original draft, writing – review & editing.
- Christian Deramond *Gálvez: investigation, methodology, data curation, formal analysis, software, writing – original draft, writing – review* & editing.
- *Ángela Machuca: resources, supervision, visualization, writing – review* & editing.
- Daniel Eduardo Chávez Matamala: conceptualization, supervision, validation, project administration, resources, visualization, writing – original draft, writing – review & editing.

Ethical implications

The authors declared that all research activities were done under the framework of authorizations N° 02/2017 IX and N° 05/2022 IX.

Conflicts of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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