Inoculation methods of native strains of *Trichoderma* sp. and their effect on the growth and yield of quinoa

Métodos de inoculación de cepas nativas de *Trichoderma* sp. y su efecto sobre el crecimiento y rendimiento de la quinua

Métodos de inoculação de cepas nativas de *Trichoderma* sp. e seu efeito no crescimento e rendimento da quinoa

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ABSTRACT

The use of endophytic fungi is an effective alternative to control pathogens, improve plant metabolism and yield in crops. The objective of this study was to assess the effect of five different strains of *Trichoderma* sp. on the growth and yield of quinoa plants (*Chenopodium quinoa* Willd) by using two methods of inoculation: a) pelleted seed, and b) drenching with the endophytic fungi. A completely randomized design with a 2 x 5 factorial arrangement, plus a control with five repetitions was used. The 11 treatments were evaluated with five repetitions. Yield, and aerial and root growth variables were determined. There were no interactions between strains and inoculation methods for aerial plant growth, but there were for root growth and yield. The seed pelleting method produced a higher aerial growth compared to the drench method. In root length, the greatest values were found with the TE-7 and TE-126 strains combined with the pelleted seed method. Likewise, the TE-126 strain induced the greatest dry biomass of roots using the same method. The yield varied between 4147.6 and 3222.7 $kg.ha^{-1}$ in most of the strain-method combinations, without significant differences between them. Statistically, the control always ranked last, indicating the importance of the seed inoculation. *Trichoderma* sp. produced increases in vegetative growth and quinoa yield, with TE-7 and TE-126 being the best strains. Furthermore, seed pelleting promoted vegetative growth of the plants, while grain yield was not affected by the inoculation method.

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Resumen

El uso de hongos endófitos en los cultivos es una alternativa efectiva para el control de patógenos, mejorar el metabolismo vegetal y su rendimiento. El objetivo de este estudio fue evaluar el efecto de diferentes cepas de *Trichoderma* sp. sobre el crecimiento y rendimiento de la quinua (*Chenopodium quinoa* Willd), mediante el empleo de dos métodos de inoculación: a) semilla peletizada, y b) aplicación del hongo al suelo en suspensión acuosa (vía *drench*). Se usó un diseño completamente al azar con arreglo factorial de 2 x 5, más un testigo, con cinco repeticiones. Se determinaron variables de crecimiento aéreo y radical, y el rendimiento. No hubo interacción entre cepas y métodos de inoculación para el crecimiento aéreo, pero sí para el crecimiento de raíces y rendimiento. La peletización de semillas produjo un mayor crecimiento aéreo respecto al método vía *drench*. La mayor longitud de raíces se obtuvo con las cepas TE-7 y TE-126, combinadas con el método de semilla peletizada, así como, la mayor biomasa seca de raíces con la cepa TE-126. El rendimiento varió entre 4147,6 y 3222,7 $kg.ha^{-1}$ en la mayoría de las combinaciones cepa-método, sin diferencias significativas entre ellas. Estadísticamente, el testigo ocupó siempre el último lugar, indicando la importancia de la inoculación de la semilla. *Trichoderma* sp. produjo incrementos en el crecimiento vegetativo y rendimiento de la quinua, siendo TE-7 y TE-126 las mejores cepas. Por su parte, el peletizado de la semilla promovió el crecimiento vegetativo de las plantas, mientras que el rendimiento en grano no fue afectado por el método de inoculación.

Palabras clave: Crecimiento, rendimiento, producción, hongos endófitos, *Chenopodium quinoa.*

Resumo

O uso de fungos endofíticos em cultivos é uma alternativa eficaz para o controle de patógenos, melhorando o metabolismo e a produtividade das plantas. O objetivo deste estudo foi avaliar o efeito de diferentes cepas de *Trichoderma* sp. sobre o crescimento e produtividade da quinoa, *Chenopodium quinoa* (Willd), por meio de dois métodos de inoculação: a) semente peletizada e b) aplicação do fungo ao solo em suspensão aquosa (via encharcamento). Utilizou-se o delineamento inteiramente casualizado com arranjo fatorial 2 x 5, mais uma testemunha, com cinco repetições. Variáveis de crescimento aéreo e radicular e produtividade foram determinadas. Não houve interação entre linhagens e métodos de inoculação para crescimento aéreo, mas houve para crescimento radicular e produtividade. A peletização das sementes produziu um maior crescimento aéreo em relação ao método por imersão. O maior comprimento de raiz foi obtido com as linhagens TE-7 e TE-126 combinadas com o método de sementes peletizadas. Da mesma forma, a linhagem TE-126 produziu a maior biomassa seca de raízes neste mesmo método. A produtividade variou entre 4147,6 e 3222,7 $kg.ha^{-1}$ na maioria das combinações estirpe-método, sem diferenças entre elas. Estatisticamente, o controle sempre ficou em último lugar, indicando a importância da inoculação das sementes. *Trichoderma* sp. produziu aumentos no crescimento vegetativo e na produção de quinoa, sendo TE-7 e TE-126 as melhores linhagens. E por sua vez, a peletização da semente promoveu o crescimento vegetativo das plantas, enquanto a produtividade de grãos não foi afetada pelo método de inoculação.

Palavras-chave: Crescimento, rendimento, produção, fungos endofíticos, *Chenopodium quinoa.*

Introduction

Quinoa (*Chenopodium quinoa* Willd) is considered a functional food, of great nutritional value, and an alternative for food security worldwide (FAO, 2016; Alandia *et al*., 2020), due to the balance of its essential amino acids, fatty acids, vitamins, and minerals (García *et al*., 2015). Belonging to the family Amaranthaceae, this dicot native to the Andean region of Peru and Bolivia has been widely cultivated by pre-Columbian cultures (FAO, 2016; Veas and Cortés, 2018).

In Peru, quinoa has had substantial growth in production and exports, with a higher competitive profitability than other traditional highland crops. At the end of 2021, production was reported at 106,643 t (INEI, 2022), with a variation of 6.4 % over the previous year and cumulative export volumes for October 2021 of 41,600 t (MINAGRI, 2021).

In Peru, even though the commercial and nutritional value of quinoa is recognized, it is still grown under traditional conditions with limited yields. The INIA Salcedo variety is one of the most widely used materials and is used as a standard for comparison when evaluating other varieties of the crop (Urdanegui *et al*., 2021).

The tendency to produce quinoa with export qualities suggests the use of cultural practices that allow this purpose to be achieved efficiently, without resorting to the use of insecticides and chemical fertilizers. One way to promote growth in a sustainable manner is through direct interaction between beneficial microbes and the host plant, and indirectly through their antagonistic activity against pathogens (Berg, 2009), with the *Trichoderma* genus being one of the most important beneficial fungi.

Interactions with *Trichoderma* induce higher plant yields due to improvements in the availability of nutrients in the soil, healthy root development (Chagas *et al*., 2017), and changes in root microflora composition (Harman and Shoresh, 2007). Its rapid mycelial growth and production of extracellular enzymes, as well as antibiotics and antifungals, allow it to compete against pathogenic fungi, while its spores contribute to the colonization of diverse substrates (Benítez *et al*., 2004). The fungus has the ability to adhere, recognize roots, penetrate and resist toxic metabolites produced, as a sign of its potential for agricultural sustainability through biocontrol, biostimulation, and biofertilization (Baron and Rigobelo, 2022).

Trichoderma can be found in different ecosystems and soils of agricultural use, however, when it is incorporated into the soil its population increases and delays the establishment of pathogenic microorganisms. One way to apply it is by using the drench method, a technique that consists of applying the antagonistic fungus suspended in water on the soil surface, where the seeds and absorbent roots are located (Loli, 2012).

On the other hand, beneficial effects have been demonstrated when *Trichoderma* is added directly to seeds. One way to achieve this, is by pelleting, which consists of coating the seeds with microorganisms of high biochemical potential for nutrient assimilation (Vergani and Zúñiga, 2018), or with inert powders and a cementing agent to homogenize their shape and size and provide rigidity (Gaviola, 2020).

In this sense, pelleting with biofertilizers represents a useful mechanism for the restitution of nutrients to soils with the consequent improvement in crop yields from small seeds (Afzal *et al*., 2020).

Considering the beneficial effects of this fungus in this study, the effect of five strains of *Trichoderma* sp. applied by two different inoculation methods on the growth and yield of quinoa under greenhouse conditions was evaluated.

Materials and methods

The research was carried out in the Phytopathology Laboratory and the greenhouse of the Professional School of Agronomic Engineering of the National University of the Altiplano of Puno, located in the province of Puno, Peru, at an altitude of 3,824 meters above sea level. During the greenhouse phase, between January and July, the average minimum and maximum temperatures were 7.1 and 27.4 °C, respectively.

Biological material

Quinoa seeds of the Salcedo INIA variety were used, with 11 % humidity, varietal purity of 99.1 %, and germination power of 98 %. Five native strains of *Trichoderma* sp. were used, which were provided by the same laboratory and obtained from leaves, stems and rhizosphere of quinoa plants, and stored at -10 °C in 2 mL cryogenic tubes, with 20 % ($^v/_v$) glycerin (Robles *et al*., 2016).

The strains were reactivated in Petri dishes with PSA (potato, sucrose, and agar), and inoculated at 25 °C for five days; later, they were multiplied on plates with the same medium (León *et al*., 2018). Subsequently, *Trichoderma* spore reproduction was performed with the use of techniques and procedures established in the Phytopathology Laboratory of the National University of the Altiplano, which consisted of placing 400 g of rice substrate in polyethylene bags of 0.4 L capacity, 150 cm^3 of 3 % calcium bicarbonate was added; it was homogenized and sterilized at a temperature of 120 °C for 20 minutes. In each bag, pieces of mycelium and 50 % of the culture medium contained in the plates were placed and taken to an incubator, with lighting and a temperature of 25 °C for 14 days (Arévalo *et al*., 2017).

Inoculation methods

For the inoculation of the *Trichoderma* sp. strains, a suspension of conidia was used at a concentration of 1 x 10^7 $CFU^{-1}.cm^{-3}$, and was carried out by two methods: seed pelleting and application to the substrate using the drench method. For the first method, each seed was mixed with the conidia suspension one day before sowing. For the second method, at the time of sowing, 250 cm^3 of the suspension were applied in each propagation bag according to the methodology established in the laboratory.

The substrate used for sowing was obtained by mixing agricultural soil, organic matter, and sand. The agricultural soil was previously disinfected by solarization, in beds of 5-10 cm and moistened with water until it reached its field capacity, and it was covered with plastic for one month. The materials were mixed in a 2:1:1 ratio and then placed in polyethylene bags (4 L capacity). Three days before sowing, the substrate was watered with water up to its maximum capacity. Three seeds were sown per bag, and after emergence the most vigorous seedling was left.

Biostimulation trial

Each treatment consisted of a combination of the five strains and the two inoculation methods. In addition, a control treatment was used, without inoculation, for a total of 11 treatments (table 1).

Table 1. ***Trichoderma*** **sp. strains evaluated by two inoculation methods on quinoa seeds of the Salcedo INIA variety at the National University of the Altiplano, province of Puno, Peru.**

Treatment	*Trichoderma* sp strain	Inoculation method
1	TE-5	Drench method
2	TE-7	Drench method
3	TE-3	Drench method
4	TE-55	Drench method
5	TE-126	Drench method
6	TE-5	Pelleted seed
7	TE-7	Pelleted seed
8	TE-3	Pelleted seed
9	TE-55	Pelleted seed
10	TE-126	Pelleted seed
11	Control	Without inoculation

Plant height, stem diameter, number of leaves, aerial dry biomass, root length, root dry biomass, and yield were evaluated at 188 days after sowing (dds). Root samples were collected at the beginning of flowering (89 dds). They were washed, and the dry mass and length were determined using the Asses 2.0 image analyzer program. Crop yield was estimated considering grain weight and trial sowing area, to convert to kilograms per hectare. The aerial and root dry biomass were determined from the fresh and dry weight, obtained by drying them in an oven at 60 °C for three days.

Statistical analysis

The study was carried out following a completely randomized design with five repetitions, and a 2 x 5 factorial arrangement, combining the two inoculation methods and the five *Trichoderma* sp. strains, plus a control treatment to form a total of 11 treatments. Data were subjected to analysis of variance and Tukey's test ($p \leq 0.05$) using the SAS version 9.2 statistical package (Cary, NC, USA).

Results and discussion

Vegetative growth

Seed pelleting with *Trichoderma* sp. strains promoted a greater increase ($p \leq 0.05$) in aerial growth variables, i.e., plant height, number of leaves, stem diameter and aerial dry biomass, compared to the drench method to soil (table 2). For these variables, no interaction was detected between the strains and the inoculation method used; it was noted that all treatments that received the fungus strains exceeded the control treatment. Among the strains evaluated, TE-7 showed superiority or similarity with the rest of the strains for all aerial growth variables.

The effectiveness of *Trichoderma* sp. strains to favor the germination and emergence of seedlings has been demonstrated by other authors (Banjac *et al*., 2021). In the present study, it was demonstrated that treatments containing *Trichoderma* sp. strains also favored subsequent seedling development under greenhouse conditions. It is inferred that the growth of plants from pelleted seeds had favorable conditions against the attack of microorganisms in the early stages, due to the antagonistic effect of *Trichoderma*; in addition to the availability of moisture and nutrients due to the presence of the fungus.

Table 2. Vegetative and root growth of quinoa seedlings var. Salcedo INIA at 188 dds after the application of different strains of *Trichoderma* sp. by two inoculation methods.

Inoculation methods	Plant height (cm)	Stem diameter (mm)	Number of leaves	Aerial dry biomass (g.plant^{-1})
Drench method to soil	117.27 b	9.05 b	99.43 b	6.72b
Pelleted seed	128.53 a	9.62 a	111.57 a	7.54 a
***Trichoderma* sp. strains**				
TE-3	128.50 a	9.66 ab	90.90 b	7.31 a
TE-5	125.60 a	9.31 abc	93.50 b	8.20 a
TE-7	127.50 a	9.88 a	115.60 a	7.10 ab
TE-55	122.70 ab	8.98 bc	98.00 ab	6.99 ab
TE-126	123.00 ab	9.66 ab	94.50 ab	7.16 ab
Control	110.10 b	8.54 c	87.00 b	6.02 b
CV (%)	8.67	6.63	16.84	13.03

dds: days after sowing. Different letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

Stocco *et al*. (2019), mentioned that the seed coating technique has potential advantages since it favors rapid and uniform seedling growth. This could explain why seed pelleting with the fungus produced higher values in aerial growth variables when compared to the application of the drench method.

López-Valenzuela *et al*. (2019), obtained higher growth in plant height, stem diameter, root volume, and foliage dry weight in maize plants when seeds were impregnated with a concentration of *Trichoderma* spp. similar to that of the present study (1 x 10^7 spores^{-1}.cm^{-3}).

Comparative results have been found in different studies. For example, Yaqub and Shahzad (2008) found that inoculation of sunflower (*Helianthus annuus* L.) and mung bean (*Vigna radiata* (L.) Wilczek) seeds with *Trichoderma* spp. conidia in water or in a sugar solution promoted a significantly higher plant height in relation to the control.

Length and dry biomass of roots

In general, strain TE-7 followed by TE-5 and TE-126 statistically outperformed ($p \leq 0.0001$) the rest of the treatments in relation to plant root length (Table 3A). In this same variable, the inoculation method using pelleted seed outperformed the drench method. Likewise, when comparing the interactions, it was observed that when pelleted seeds were used, strains TE-7 and TE-126 produced the highest values in root length in relation to the rest ($p \leq 0.05$), regardless of the inoculation method (table 3B).

For its part, the control was outperformed by all strain x method combinations.

When considering the main effects, no differences were detected between the strains in relation to root dry biomass; however, among the methods, the use of pelleted seed was statistically superior (table 4A). On the other hand, the interaction effect showed that the greatest root dry biomass was produced with the use of strain TE-126 in combination with the use of pelleted seed, surpassing TE-3 inoculated with this same method and with no differences with TE-3 and TE-126 inoculated with the drench method (Table 4B). In all cases, the control treatment was significantly inferior.

Table 3. Root length (cm) in quinoa seedlings of Salcedo INIA variety at 89 dds after the application of different strains of *Trichoderma* sp. by two inoculation methods.

A) Main effects.

Trichoderma sp. strains					
TE-5	TE-7	TE-3	TE-55	TE-126	Control
46.07 b	48.32 a	40.11 d	43.16 c	45.40 b	29.67 e

Inoculation methods	
Drench method to soil	Pelleted seed
41.70 b	47.53 a

B) Interactions

Inoculation methods	*Trichoderma* sp. strains					
	TE-5	TE-7	TE-3	TE-55	TE-126	Control
Drench method to soil	45.20 bc	43.92 c	39.00 e	41.05 d	39.30 de	29.67 f
Pelleted seed	46.93 b	52.71 a	41.19 d	45.27 bc	51.47 a	

dds: days after sowing. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

Table 4. Dry biomass of roots in quinoa var. Salcedo INIA at 89 dds after the application of different strains of *Trichoderma* sp. by two inoculation methods.

A) Main effects.

Trichoderma sp. strains					
TE-5	TE-7	TE-3	TE-55	TE-126	Control
1.201 a	1.053 a	1.049 a	1.158 a	1.231 a	0.784 b

Inoculation methods	
Drench method to soil	Pelleted seed
1.017 b	1.142 a

B) Interactions.

Inoculation methods	*Trichoderma* sp. strains					
	TE-5	TE-7	TE-3	TE-55	TE-126	Control
Drench method to soil	1.039 bcde	1.002 cde	1.822 abcd	1.014 cde	1.068 abcde	0.784 e
Pelleted seed	1.362 ab	1.104 abcde	0.915 de	1.300 abc	1.394 a	

dds: days after sowing. Different letters indicate significant differences according to Tukey's test (p ≤ 0.05).

The results show that root growth, both in length and biomass, was favored by the use of pelleted seed, particularly with strain TE-126, which produced the highest values for both variables. It is likely that seed pelleting with this strain has produced from germination, a greater antagonistic effect against pathogens and improved soil conditions, close to the first emerged roots.

They also indicate that the strains of *Trichoderm*a sp. evaluated have a differential effect on root growth depending on the inoculation method used. For example, in strain TE-3 the highest value of root biomass was found when it was inoculated with the drench method but the lowest value when pelleted seed was used.

Similarly, Ruíz-Cisneros *et al*. (2018), obtained tomato plants with greater root length and dry weight when different *Trichoderma* isolates were applied, with respect to the control treatment, and Camargo-Cepeda and Avila (2014), found a 68 % increase in root dry weight in *Pisum sativum*, with respect to the control treatment, after inoculating seed and soil with commercial strains of *Trichoderma*. For their part, Brenes-Madriz *et al*. (2019) found the highest elongation and dry biomass of the root, after applying *T. asperellumn* in sweet pepper plants (*Capsicum annuum* L.).

Growth promotion occurs when *Trichoderma* establishes a close physical association with the roots (Stewart and Hill, 2014), coupled with a change in the composition of the microflora in this organ, as well as better absorption and solubility of nutrients, formation of absorbent hairs, and deepening (Harman and Shoresh, 2007). By persisting in the soil, particularly in the rhizosphere, and eventually associated as endophytic, *Trichoderma* also generates long-term advantages (Woo *et al*., 2014).

Yield

The yield obtained in the control treatment of 1,412.6 kg.ha^{-1} (figure 1) reflects the yield usually obtained by the quinoa crop with traditional management, which fluctuates between 1.200 and 1.500 kg.ha^{-1}. The values observed in the figure highlight that the use of *Trichoderma* sp. increased the yield by two to three times the value of the control (p ≤ 0.05), which emphasizes the importance of its use in sustainable agriculture.

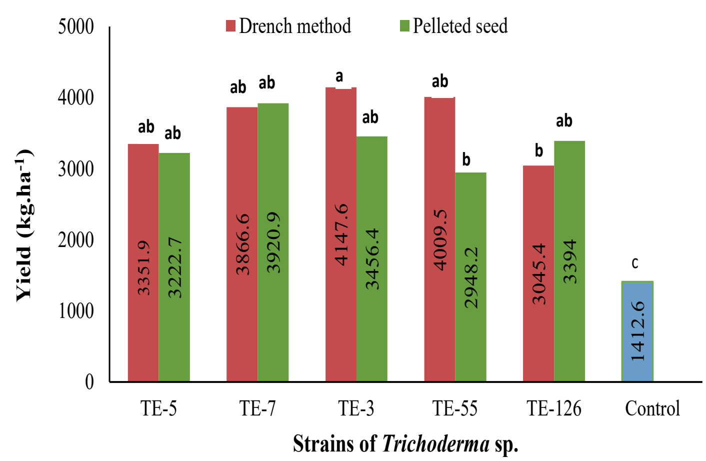

Figure 1. Yield of quinoa plants var. Salcedo INIA after the application of different strains of *Trichoderma* sp. by two inoculation methods. Different letters between columns indicate significant differences according to Tukey's test (p ≤ 0.05).

Significant differences (p ≤ 0.05) were detected for the interaction between the strains applied and the inoculation methods (figure 1), with no differences between the use of the two methods (p > 0.05). The effect of the interaction of factors on yield was observed fundamentally in the statistically lower values obtained with strain TE-126 when applied with the drench method (3,045.4 kg.ha^{-1}), or with TE-55 when seed pelleting was used (2,948.2 kg.ha^{-1}). The interaction between strain TE-3 and its application to the soil drench method, produced the highest yield in quinoa plants (4,147.6 kg.ha^{-1}), but without significant differences with most of the remaining strains.

The results obtained agree with those of De Oliveira *et al*. (2018), who after applying suspensions of *T. harzianum* and *T. asperellum* to the soil, managed to increase wheat yield 110 days after sowing under greenhouse conditions.

In the present study, it is observed that the control treatment showed the lowest yield and was outperformed in all cases and with both inoculation methods when compared to treatments in which *Trichoderma* was included. Ruíz-Cisneros *et al*. (2018), found that *T. longibrachiatum* strains applied to the substrate produced the highest tomato yields (*Solanum lycopersicum),* higher than 240 g per plant, compared to the control plants. On the contrary, Brenes-Madriz *et al.* (2019) when applying *T. asperellum* did not observe significant differences between treatments on sweet pepper yield (*C. annuum* L.) under greenhouse conditions. El-Ibrahime and Mourad (2020), found that the application of *T. viride* using the drench method to the soil produced lower yields in sunflowers compared to the foliar application of *T. harzianum;* however, both treatments were able to outperform the control.

Concerning quinoa, León-Ttacca *et al*. (2021) found that *Trichoderma* sp. acted as an aggressive mycoparasite, and promoted a higher yield of quinoa, a condition that possibly helped to enhance the positive effect on yield observed in the present work. Infante *et al*. (2009), pointed out that the more *Trichoderma* can manifest diverse modes of action, the more efficient and lasting can be its favorable effect on the crop.

The benefits that *Trichoderma* produces in the plant have been widely reported in the literature, so the promotion of higher levels of aerial and root development are predisposing conditions for high yields, as was observed in the quinoa plants evaluated in this research. Likewise, the beneficial use of seed pelleting is highlighted, which was evident in the response of practically all the variables evaluated.

Conclusions

The seed pelleting method with *Trichoderm*a sp. strains is more effective than the drench method to promote the vegetative growth of quinoa. It is found that strain TE-7 generates the highest growth in plants grown under greenhouse conditions. The grain yield is significantly increased by the application of *Trichoderma* sp., independently of the inoculation method.

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