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Research Article

Evaluation of Growth Promotion and Pathogenicity of Endophytic Fungi from the Root of *Chenopodium Quinoa* Willd

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Abstract

Symbiotic associations between endophytic fungi and *Chenopodium quinoa* have been reported to be beneficial for increasing tolerance to drought and soil salinity, being this the only crop grown in the Southern Altiplano of Bolivia. The symbiotic interaction of endophytic fungi was investigated, considering their detrimental and mutualistic effects. 38 strains of endophytic fungi from the fungal collection of the Faculty of Agronomy were used. The percentage of germination and root length were measured in vitro (4 days). The percentage of emergence (7 days) and plant height (14 days) in pots were also evaluated. The fungi that did not impair germination were strains VP42, VP44, *Alternaria* sp. VP37, *Fusarium* sp. VP05 and *Fusarium* sp. VP30. Fungi that stimulated a significant increase in radicle longitudinal growth were *Fusarium* sp. VP35, *Alternaria* sp. VP37 and strain VP18. Fungi that caused a high mortality rate during the emergence phase were strain VP01, *Alternaria* sp. VP15, *Fusarium* sp. VP02, *Fusarium* sp. VP07, *Fusarium* sp. VP08, *Fusarium* sp. VP12, *Fusarium* sp. VP23 and *Fusarium* sp. VP36. The endophytic strains *Alternaria* sp. VP37, *Fusarium* sp. VP35 and strain VP18 stimulated superior seedling growth. The present research work reveals that some endophytic fungi of the *Alternaria* and *Fusarium* genera can behave as pathogens during the germination stage, while others have the function of promoting quinoa growth.

Keywords

Alternaria, *Fusarium*, Endophytic Fungus, Pathogenesis, Quinoa

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.), is an annual dicotyledonous plant belonging to Amaranthaceae family [1]. Currently, it is cultivated in more than 125 countries around the world, due to its genetic diversity of seeds preserved by generations of farmers in the Andes [2]. It is the only crop adapted for thousands of years to the adverse conditions of the Southern Altiplano of Bolivia [3, 4].

Plant-fungus interactions date back 400-500 million years [5-7]. Plants do not thrive isolated, as constantly interact closely with various microorganisms, mainly bacteria and

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fungi [8-11]. Their symbiotic relationship with plants attracts attention for its benefits in agriculture.

Fungi benefits to their host, existing a wide variety of plant-fungal symbiotic interactions [12-15]. Favorable interactions, for example, allow plants to adapt to various ecosystems [16-19]. For quinoa, endophytic fungi (EF) perform various functions that improve its resistance to adverse conditions, mitigating abiotic stress [20, 21].

Associations can be beneficial [8, 22-25], for example, promoting the adaptation and growth of plants in extreme conditions [26]. Most plants interact with microorganisms [27, 28], EF have been reported in plants that inhabit the Arctic, Antarctic, desert, ocean, tropical forests and agricultural fields [29-33]. Growth promoting fungi can protect against plant pathogens by increasing tolerance to abiotic stress, such as drought, salinity and high temperatures through the production of secondary metabolites and hydrolytic enzymes [34-36]. This study evaluated the influence of EF inoculated in roots and its effects on germination, emergence, root development and growth promotion in quinoa. Understanding the effect of applying EF in agriculture can offer innovative solutions to increase quinoa crop productivity by mitigating the conditions of the Southern Altiplano of Bolivia.

2. Materials and Methods

2.1. Study Area

The trials were carried out at the Plant Science Laboratory of the Faculty of Agronomy of the Technical University of Oruro. The HE strains used in this study were partially identified and are part of the mycological collection of the Faculty of Agronomy, whose specific details are presented in Table 1.

2.2. Fungal Cultivation and Biomass Production

For *in vitro* evaluation, axenic culture of EF were prepared in Potato Dextrose Agar (PDA) medium at 25 ℃ for 14 days. Subsequently, mycelia discs (5 mm) of EF were collected [37] to evaluate their promoting or pathogenic effect on quinoa germination. For pot tests, EF biomass was produced in 250 mL flasks inoculating 10 mycelia discs in 50 g of sugarcane bagasse.

2.3. In Vitro Germination and Root Length

Seeds of quinoa (Pandela rosada variety) were disinfested according to González-Teuber et al. [21]. *In vitro* evaluation of EF effect on quinoa germination and root length, consisted of applying a mycelia disk in a 100 mm Petri dish with Murashige and Skoog (MS) medium (38 HE with 3 replicates + uninoculated Petri dishes) along with 10 desinfested quinoa seeds located around the disk. Petri dishes were incubated at 16 ℃ for 4 days, with daily monitoring. Finally, germination (%) and root length (cm) were evaluated after the incubation period [38, 39].

Table 1. *Endophytic fungi in the current study.*

Specimens	Strain code	Host	Place
Alternaria sp.	VP15	Quinoa root	Southern Alti-plano - Bolivia
Alternaria sp.	VP24	Quinoa root	Southern Alti-plano - Bolivia
Alternaria sp.	VP37	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP12	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP11	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP16	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP02	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP21	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP22	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP23	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP03	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP30	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP35	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP36	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP39	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP40	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP41	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP43	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP05	Quinoa root	Southern Alti-plano - Bolivia
Fusarium sp.	VP06	Quinoa root	Southern Alti-plano - Bolivia

Specimens	Strain code	Host	Place
Fusarium sp.	VP07	Quinoa root	Southern Altiplano - Bolivia
Fusarium sp.	VP08	Quinoa root	Southern Altiplano - Bolivia
Penicillium sp.	VP32	Quinoa root	Southern Altiplano - Bolivia
Penicillium sp.	VP38	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP01	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP14	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP18	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP19	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP20	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP28	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP29	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP31	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP33	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP34	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP04	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP42	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP44	Quinoa root	Southern Altiplano - Bolivia
Unknown	VP09	Quinoa root	Southern Altiplano - Bolivia

2.4. Pots Assay: Emergence and Plant Length

Inoculation of EF in pot experiments consisted of: i) 15 g of sugarcane bagasse colonized with the fungus was placed at the bottom, ii) 600 g of sterile soil (collected from fallow quinoa plots in Southern Altiplano, commonly sandy) into 1 L plastic pot (38 pots with 3 replicates + uninoculated pots). Then, all pots were covered with sterile plastic bags until sowing. 10 disinfested quinoa seeds were sown at the surface and covered again with sterile plastic bags until germination. The pots were kept at room temperature (14 ℃) with relative humidity of 37%. The emergence percentage (%) was evaluated at 7 days and the plant length (cm) at 14 days after sowing.

2.5. Statistical Analysis

A complete randomized design (CRD) was used for the *in vitro* evaluation and a complete randomized block design (RBD) for the pots assay. Analysis of variance (ANOVA) was determined using R 4.2.3 software (R Development Core 2023) and Tukey test was used post-hoc.

3. Results

In vitro assay. There were significant differences in germination of quinoa seeds subjected to 38 EF ($p<0.05$). EF such as *Alternaria* sp. VP37, *Alternaria* sp. VP24, *Fusarium* sp. VP05, *Fusarium* sp. VP30, strains VP44 and VP42 applied to quinoa seeds promoted 100% of germination. In contrast, EF as *Alternaria* sp. VP15, *Fusarium* sp. VP12, *Fusarium* sp. VP23, *Fusarium* sp. VP02, *Fusarium* sp. VP08, *Fusarium* sp. VP07, strain VP01 and *Fusarium* sp. VP36 inhibited the germination completely, but *Fusarium* sp. VP21, strain VP14, *Fusarium* sp. VP43, *Fusarium* sp. VP39, strain VP31, *Fusarium* sp. VP22 and *Fusarium* sp. VP16 inhibited 93.33% of germination. Other strains showed intermediate values (Figure 1). On the other hand, there were significant differences in root length among the 38 EF ($p<0.05$). Growth promoting fungi based on longitudinal development of roots were *Fusarium* sp. VP35, *Alternaria* sp. VP37 and strain VP18 that reached 49.85, 49.73 and 48.85 mm respectively, followed by *Alternaria* sp. VP24, *Fusarium* sp. VP05, strains VP29 and VP42 and VP44 that promote relatively similar quinoa roots varying from 46.43 to 46.12 mm. In contrast, the strain VP14 and *Fusarium* sp. VP43 affected root development with 23.28 and 21.98 mm, respectively (Figures 2, 3).

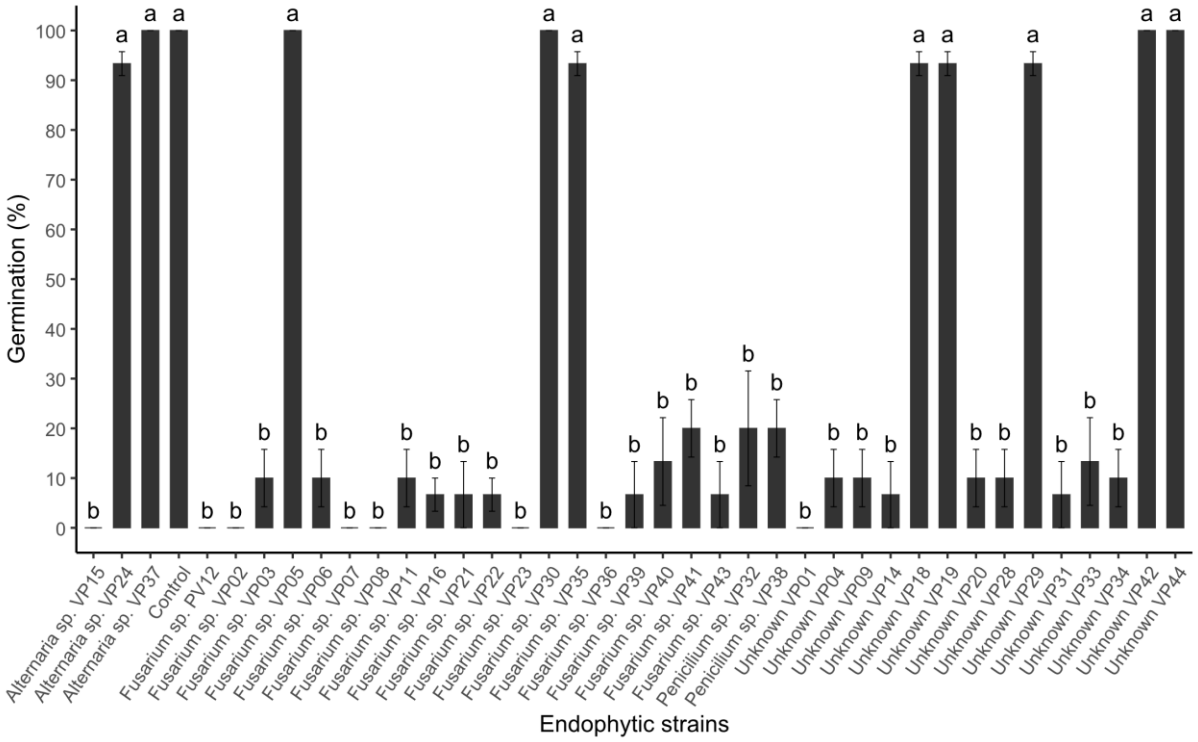

Figure 1. *Quinoa germination can be influenced by inoculation with endophytic fungi.*

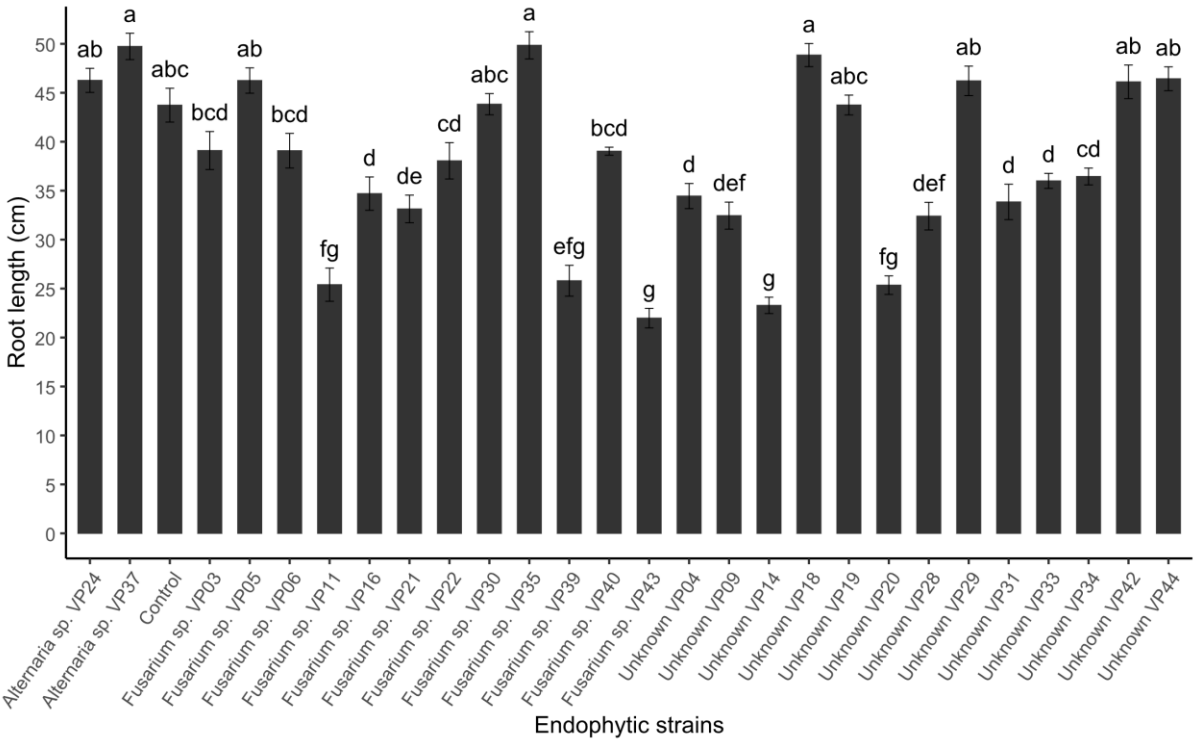

Figure 2. *Root development of quinoa can be influenced by inoculation with endophytic fungi.*

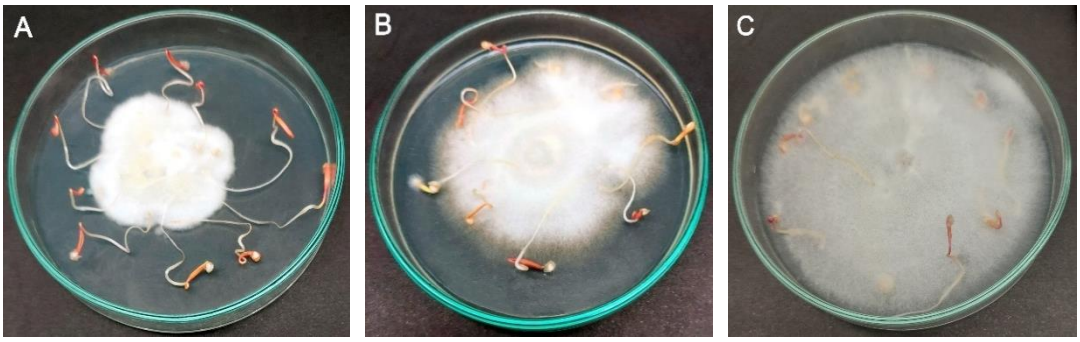

Figure 3. *In vitro test: Endophytic fungi effect on quinoa root length. (A) Fungus observed to be beneficial as it promotes root growth, (B) Fungi has a moderately detrimental effect on root development, and (C) certain endophytic fungi are detrimental to root development.*

Emergence and height of potted plants. Fully emerged cotyledons were statistically different in the presence of EF ($p<0.05$). The endophytic fungi that did not cause damage were strains VP18, VP19, VP29, VP42, VP44, *Fusarium* sp. VP35, *Alternaria* sp. VP37, *Fusarium* sp. VP05, *Alternaria* sp. VP24 and *Fusarium* sp. VP30, which only limited emergence by 3.33%. EF that presented less emergence of cotyledons and caused high mortality were *Alternaria* sp. VP15, *Fusarium* sp. VP07, *Fusarium* sp. VP08, *Fusarium* sp. VP02, *Fusarium* sp. VP12, *Fusarium* sp. VP36, strain VP01 and *Fusarium* sp. VP23 with 96.67% (Figure 4).

Plant height was statistically significant ($p<0.05$). Endophytic strains *Alternaria* sp. VP37, *Fusarium* sp. VP35 and strain VP18 promoted higher plant development reaching 55.98, 54.95 and 53.58 mm respectively, followed by strains VP44, VP42, VP19 and *Fusarium* sp. VP05 with developments 44.91, 44.86, 41.52 and 41.62 mm, respectively. *Fusarium* sp. VP03, *Fusarium* sp. VP06, as well as strains VP04, VP31 and VP33, showed a development similar to that of plants free of infection by endophytic fungi (control). Other endophytic strains had negative effects on plant height compared to the control (Figures 5, 6).

4. Discussion

Some EF from quinoa roots have positive effects on germination, root length, emergence and plant height. However, the vast majority caused detrimental effects including death. Several studies have described the positive effect of the association between EF and several crops [32, 40-46]. The HEs that were isolated from the quinoa root of the Atacama Desert, had their positive effect on the morphological and physiological characteristics of quinoa by inoculation of *Talaromyces minioluteus* and *Penicillium murcianum* under drought and salinity conditions [20, 46].

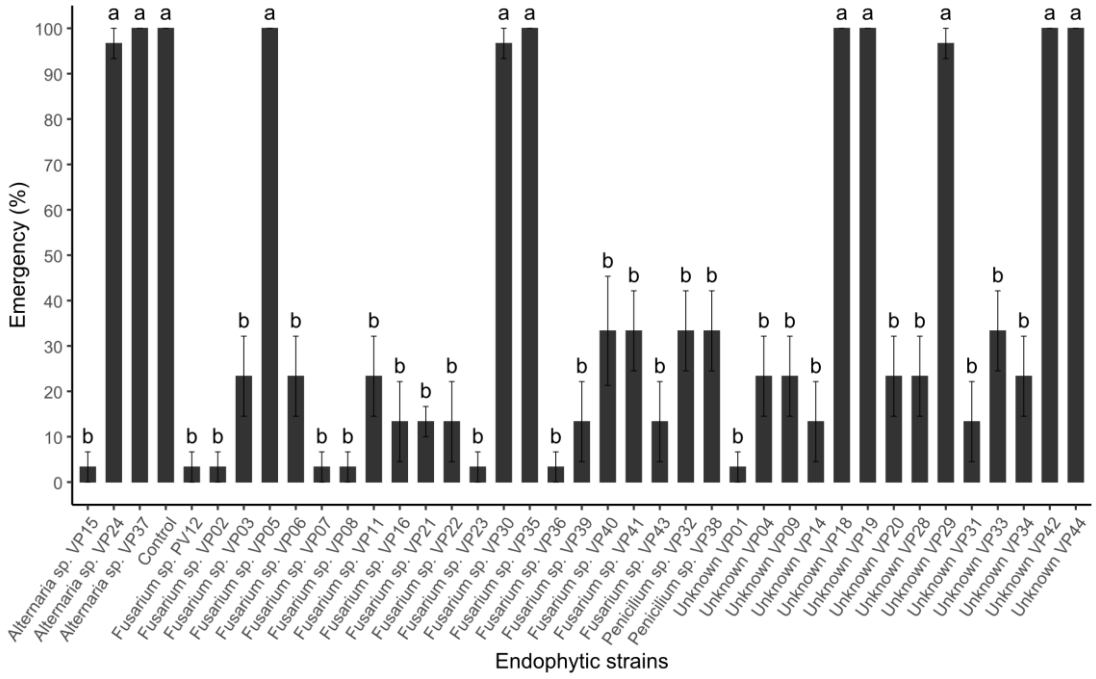

Figure 4. *Effect of inoculation with endophytic fungi in the emergence phase of quinoa.*

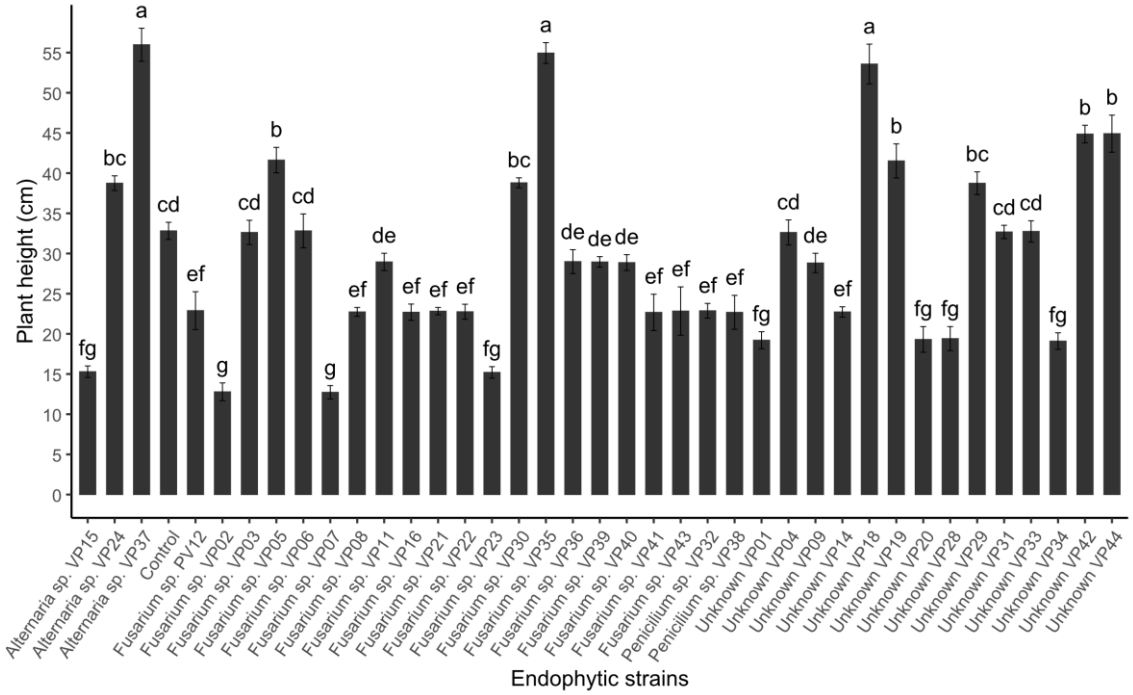

Figure 5. *The effect of inoculation with endophytic fungi on the height growth of quinoa plants.*

EF from quinoa root such as *Alternaria* sp. VP37, *Fusarium* sp. VP05, *Fusarium* sp. VP30, strains VP44 and VP42 promoted 100% of *in vitro* (MS and PDA medium) germination compared to other endophytes. Moreover, under controlled conditions in pots assay, strains *Fusarium* sp. VP35, *Fusarium* sp. VP05, *Alternaria* sp. VP37, strains VP18, VP19, VP42 and VP44 promoted 100% of plant emergence (Figure 4). The symbiosis between EF and plants can increase or reduce certain phytohormones, for example, by secreting indole acetic acid (IAA) and gibberellic acid (GA) that affect longitudinal growth of roots and phylosphere [47].

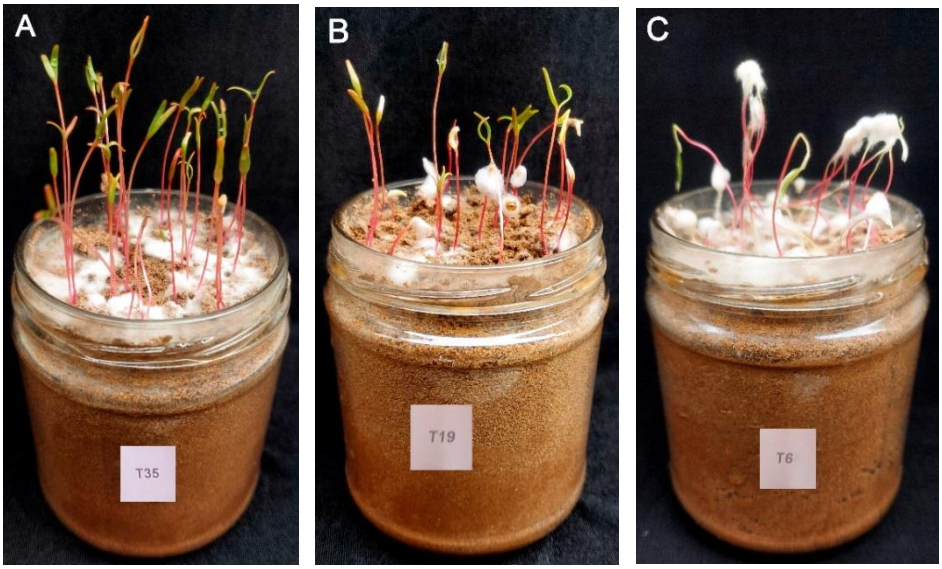

Figure 6. *Impact of endophytic fungi inoculation on quinoa growth. In (A), a positive boost in development is evident due to the mutualistic strain. On the other hand, in (B), it is observed that the endophytic fungus has a moderate detrimental effect, while in (C), it is shown that this strain is highly detrimental due to its pathogenic behavior.*

Three EF that promoted greatest root elongation were *Fusarium* sp. VP35, *Alternaria* sp. VP37 and strain VP18, reaching a plant height of 55.98, 54.95 and 53.58 mm (Figure 5). These results reveal that fungi of the *Fusarium* genus may

promote plant growth, probably by producing IAA, nitrogen fixation, phosphate solubilization and production of siderophores such as *F. annulatum* and *F. proliferatum* [48]. Nevertheless, the relationship between plant-fungus can be beneficial and/or pathogenic [49], for example, those symbionts that do not cause any disease symptoms can colonize host plants tissues and there are benefits for both parties [50]. In contrast, harmful aspects are described to interfere in transport of nutrients and water [51], abnormal metabolism in different stages of plant growth [52]. These negative effects can be controlled by other EF [53], for example, *A. fumigatus* that has the ability to control pathogens by producing aflatoxins [54]. Based upon the above described, the results of the initial vegetative development in this study showed that EF can be harmful and/or beneficial on the development of quinoa (Figure 6). For example, the strains *Alternaria* sp. VP37, *Fusarium* sp. VP35 and strain VP18 promoted seedling growth, but *Fusarium* sp. VP02 and *Fusarium* sp. VP07 are the strains that affected longitudinal elongation of the seedlings. In root development, 25.92% of EF promoted growth and the rest restricted elongation. These results are similar to those of other authors such as [55] who reported that there are pathogenic fungi of the genus *Penicillium*. In contrast, there are studies that highlighted how EF stimulate the root growth to promote nutrients absorption [44], in exchange the plants synthesize several hydrolytic enzymes such as xylanase, pectinase, cellulase and proteinase that favor the fungi to access plant tissues [32]. Understanding the dynamics of interaction between endophytic fungi and crop plants is essential to take advantage of the positive effects and mitigate the risks of potential pathogenic effects. That is essential for the development of agricultural management strategies that optimize the use of endophytic fungi in the improvement of crops such as quinoa, especially under abiotic stress conditions, such as drought and salinity.

5. Conclusion

Some strains of EF significantly promote key processes such as germination, root growth, emergence and height development of quinoa plants. However, other strains exert inhibitory effects on these processes, which could be detrimental.

EF with growth-promoting capacity represent a resource with great potential for implementation as biostimulants in sustainable agricultural systems. However, it is imperative to carry out a rigorous and precise selection of strains before proceeding with their application in field conditions.

Abbreviations

EF	Endophytic Fungi
MS	Murashige and Skoog
PDA	Potato Dextrose Agar
IAA	Indole Acetic Acid
GA	Gibberellic Acid

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Ethical Considerations

This work was carried out according to the recommendations of related authors.

Research Limitations

There were no limitations in the present research.

Author Contributions

Victor Paco Pérez, conceived the idea and wrote the initial draft of the manuscript, *Carla Crespo Melgar* and *Fernando Pacasa Quisbert*, edited and revised the manuscript and *Edwin Marcelo Gonzales Torrico*, collaborated at microbiology laboratory.

Conflicts of Interest

The authors declare no conflicts of interest.

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