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# Mycorrhizae and Substrates in the Vegetative Propagation of Pine

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## Abstract

The effect of mycorrhizae with different substrates was evaluated on the production of (Pinus radiata D. Don.) plants in the nursery. The completely randomized experimental design (DCA) was used, with AxB factorial arrangement, with nine treatments and three repetitions, making a total of 27 experimental units (EU). Each EU had a population of 50 seedlings, making a total of 1350 in the experiment. Factor A were mycorrhizae with three levels (no mycorrhiza, native mycorrhiza, industrial mycorrhiza) and factor B were substrates, with three levels (peat + soil agricultural + sand, peat + sand + humus, mixture [peat + agricultural land + sand] + humus). The seedbed was carried out, and then after 45 days the seedlings (4-5 cm) were transplanted into 4"x7" black polyethylene bags. In native mycorrhizae, mycorrhizal fungi (Boletus edulis) were used with surface soil from pine forest; and the industrial mycorrhizae used were commercial spores (Boletus edulis). After 120 days, 18 seedlings were taken from each EU and the percentage (%) of budding, seedling height, number of leaves, root length, number of roots and % of root dry matter were evaluated. It was determined that the combination of industrial mycorrhizae with the mixture [peat + agricultural land + sand] + humus achieved significant results ($p<0.01$) in % of attachment, seedling height, number of leaves, root length, number of roots and % dry matter of roots.

**Keywords:** Pinus, mycorrhizae, peat, humus, seedling.

## 1. Introduction

The climate is undergoing a series of alterations, due to the high concentration of greenhouse gases (GHGs) in the atmosphere; the same ones that are causing changes in the climate regime (Lozano-Povis et al., 2021); as well as global warming and the increase in climate variability, with serious consequences for the balance of the planet by varying its manifestation, and indirectly on the availability of natural resources, emphasizing poverty (Intergovernmental Panel on Climate Change [IPCC], 2001 and 2007, cited by Cuellar et al., 2015 and Cuellar and Salazar, 2016). One of the strategies proposed to mitigate the effects of climate change is the retention and/or accumulation of CO2 in plant biomass, increasing carbon (C) fixation through photosynthesis (by creating or improving sinks) and reducing the rate of release of C that is already fixed in existing sinks. In this way, tropical forests and plantations, agroforestry practices, the conservation of forests in danger of deforestation, forest rehabilitation and, in general, those activities that lead to the expansion of permanent vegetation cover, can fulfill the function of C sinks (IPCC, 2003; Karsenty, 2008; Hidalgo, 2009, cited by Cuellar and Salazar, 2016), which not only contributes to mitigating climate change, but also to promoting the sustainable use of natural resources and greater well-being of rural communities (Zanabria and Cuellar, 2015).

Peru is one of the countries with the highest forest coverage (Food and Agriculture Organization of the United Nations [FAO] and National Forest and Wildlife Service [SERFOR], 2017). It has a forest area of 732,294,958 ha, distributed in low forest (73.1%), high forest (20.96%), northern dry (4.41%), Andean (0.53%), Marañón dry (0.51%) and western montane (0.18%) (Ministry of the Environment [MINAN], 2011). In the Andean area, deforestation is one of the main problems, mainly caused by climate change, indiscriminate logging and burning of forest species (Bobadilla et al, 2018; Moreira-Rivas et al., 2022). The first attempts at reforestation in Peru began with the introduction of Eucalyptus globulus in the Sierra, around 1870. Since 1988, the National Program for Watershed Management and Soil Conservation (Pronamachcs) has promoted the implementation of policies and strategies related to the management of watersheds, mainly in the high Andes, using reforestation as a component of ecosystem management (National Institute of Natural Resources [INRENA], 2005); therefore, since 1993 there has been an increase in interest in the installation of communal nurseries for the execution of reforestation projects (Vergara, 2004). The quality of forest plants depends fundamentally on the substrate used for their development; however, most nurseries have difficulty in the proper use of the substrate for the propagation of species, among which pine stands out (Sánchez, 2013; Núñez, 2022), a forest species that in recent years has been gaining ground in this process, due to the double benefit it provides (wood and production of the edible fungus (Boletus edulis) (Ancco, 2019; Vallejo & Cob, 2021), where the production of edible mushrooms has shown an average annual increase of 11% over the last decades (Romero-Arenas et al., 2019). On the other hand, the most well-known function of soil is to support and supply nutrients to plants. An important component of nutrient recycling is mycorrhizae (Pérez-Luna et al., 2019; Quintero-Gradilla et al., 2020), and it has been shown that problems in the decrease in water and nutrient uptake, low growth and survival, susceptibility to attack by some fungal diseases that cause damping off (neck and root necrosis) of seedlings in some nurseries are associated with poor mycorrhization, caused by the low density of ectomycorrhizal propagules present in the plant (Fernández-Golfin, 1996; Oliva et al., 2014; Bobadilla et al., 2018); likewise, ectomycorrhizae stand out for their symbiotic activity (Arteaga et al., 2020), they grow naturally in the secondary roots of forest trees, especially in pinaceae and in some angiosperm plants (Vergara, 2004). In this context, the purpose of the study was to evaluate the effect of native and industrial mycorrhizae with different substrates, on the production of Pinus radiata plants under nursery conditions.

## 2. Materials and methods

The research was carried out, during the months of July to December, in the Lara forest nursery in the district of Santo Tomás, province of Chumbivilcas, Cusco region, at an altitude of 3678 meters above sea level, geographical coordinates 14°30´45.6" S and 72°06´46.4" W, and UTM coordinates 8'393,608.11 N and 811,193.74 E. The area has two well-marked climatic seasons: rainy (November-March) and dry (April-October), with January being the month in which it rains with the highest intensity (156.32 mm/month). The highest temperature (21.7 °C) occurs in the month of October, while the lowest temperature (-1.0 °C) occurs in the month of July (National Meteorology and Hydrology Service of Peru [SENAMHI], 2019).

The completely randomized experimental design (DCA) was used, with factorial arrangement of AxB, with nine treatments and in three replications, making a total of 27 experimental units (UE). Each UE had a population of 50 seedlings, making a total in the experiment of 1350 (Table 1).

Board 1 Factors, levels and treatments studied

| Factor A<br>Mycorrhiza<br>Levels | Factor B<br>Substrates<br>Levels | Treatments |
|---|---|---|
| a0: No mycorrhiza | b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%) | T1(a0b0) |
| | b1: Peat moss (50%) + sand (25%)+humus (25%) | T2(a0b1) |
| | b2: Mix 60% (Peat + agricultural land + sand) + humus 40% | T3(a0b2) |
| a1: Native mycorrhiza | b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%) | T4(a1b0) |
| | b1: Peat moss (50%) + sand (25%)+humus (25%) | S5(a1b1) |
| | b2: Mix 60% (Peat + agricultural land + sand) + humus 40% | S6(a1b2) |
| a2: Industrial mycorrhiza | b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%) | T7(a2b0) |
| | b1: Peat moss (50%) + sand (25%)+humus (25%) | S8(a2b1) |
| | b2: Mix 60% (Peat + agricultural land + sand) + humus 40% | S9(a2b2) |

The work was carried out with Pinus radiata seeds acquired from the company Agro Organics Cuzco EIRL; with a purity of 98.6 % and a germinative load of 89.5 %. The storage was carried out in fine sand and covered with ichu mulching (Stipa ichu), under nursery conditions. After 45 days, the most vigorous plants were selected, with the presence of secondary roots and anaverage height of 4 to 5 cm. For the transplant (repique) 4" x 7" black polyethylene bags were used. The substrates (Factor B) were prepared according to Table 1. The population of transplanted seedlings with level $a_1$ (without mycorrhizae) was 450. For level a2 (native mycorrhizae), first, 0.405 m3 of duly moistened substrate was prepared, then mycorrhizal fungi (Boletus edulis) were extracted from a pine forest (FAO and Pronamachs, 1998; Ancco, 2019), the fruiting bodies of the fungi were crushed and the spores were mixed with the surface forest soil. 36 kg of the inoculum were taken and mixed with the substrate until homogenized, to finally carry out bagging (450) and transplantation. For level a3 (industrial mycorrhizae), first, in 500 ml of water, 100 g of mycorrhizal spores (Boletus edulis) were diluted. The roots of the seedlings were immersed in this mixture, and then transplanted into the bags (450), with the corresponding substrate. Weed control was manual and watering was frequent (50 and 70 % relative humidity in the nursery).

At 120 days, 18 seedlings were taken from each experimental unit and the percentage (%) of engraftment, seedling height, number of leaves, root length, number of roots and % dry matter of roots were evaluated. With the corresponding data, analyses of variance (ANOVA) of each variable were performed, and when the sources of variation of the ANOVA were significant, comparisons of the means of the treatments were made using Tukey's statistical significance test (0.05).

## 3. Results

Percentage of arrest. - Significant differences ($p<0.01$) were determined in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When performing the ANOVA of the simple effects of the AxB interaction, significant differences ($p<0.01$) were determined in factor A with respect to the levels of factor B (b0, b1 and b2). Likewise, for factor B at each level of factor A (a0 and a2), no significant differences were determined for level a1 ($p>0.05$). With Tukey's test (0.05) the mycorrhizal factor presents statistical differences when combined with the levels of the substrate factor, reaching a2b2 98.00%, followed by a1b1 with 96.33% and in last place average a0b0 with 80.33% average respectively. The substrates presented statistical differences when combined with the levels of the mycorrhizal factor, achieving the highest average b2a2 with 98.00 %, followed by b1a1 with 96.33 % and in last place the combination b0a0 with 80.33% (Table 2).

Table 2 Tukey (0.05) in percentage of attachment (%), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | % | B | To | % |
| A1 | b0 | 94.68a | b2 | A0 | 87.33a |
| A2 | b0 | 87.67b | b1 | A0 | 83.67b |
| A0 | b0 | 80.33c | b0 | A0 | 80.33c |
| A1 | b1 | 96.33a | b1 | A1 | 96.33a |
| A2 | b1 | 94.00b | b0 | A1 | 94.67b |
| A0 | b1 | 83.67c | b2 | A1 | 93.67c |
| A2 | b2 | 98.00a | b2 | A2 | 98.00a |
| A1 | b2 | 93.67b | b1 | A2 | 94.00b |
| A0 | b3 | 87.33c | b0 | A2 | 87.67c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza

b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar (p>0.05)

Root length.- According to ANOVA, significant differences (p<0.01) were determined in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When performing the single-effect ANOVA of the AxB interaction, significant differences (p<0.01) in factor A were determined with respect to the levels of factor B (b0, b1 and b2). Likewise, for factor B at each level of factor A (a1 and a2), no significant differences were determined for level a0 (p>0.05.). With Tukey's significance test (0.05) for root length, it was observed that mycorrhizae show statistical differences when mixed with substrate levels, with the highest average being$_{2b2}$ with 31.45 cm,$_{2b1}$ with 30.25 cm and the combination a0b0 achieved 21.69 cm in length. For factor B (substrates) there were statistical differences when combined with the levels of mycorrhizae factor, achieving the highest average $b_{2a2}$ with 31.45 cm, followed by b2a1 with 27.28 cm and the b0a0 mixture with 21.69 cm of root length (Table 3).

Table 3 Tukey (0.05) in root length (cm), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | cm | B | To | cm |
| A2 | b0 | 25.00a | b2 | A0 | 23.17a |
| A1 | b0 | 24.36b | b1 | A0 | 22.25b |
| A0 | b0 | 21.69c | b0 | A0 | 21.69c |
| A2 | b1 | 30.25a | b2 | A1 | 27.28a |
| A1 | b1 | 26.08b | b1 | A1 | 26.08b |
| A0 | b1 | 22.25c | b0 | A1 | 24.36c |
| A2 | b2 | 31.45a | b2 | A2 | 31.45a |
| A1 | b2 | 27.28b | b1 | A2 | 30.25b |
| A0 | b3 | 23.17c | b0 | A2 | 25.00c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza

b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar (p>0.05)

Number of roots.- Significant differences (p<0.01) were determined in the number of roots in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When performing the ANOVA of the simple effects of the AxB interaction, significant differences (p<0.01) in factor A were determined with respect to the levels of factor B (b0, b1 and b2). Likewise, for factor B at each level of factor A (a1 and a2), no significant differences were determined for level a0 (p>0.05). According to Tukey (0.05) the mycorrhizal factor presents statistical differences when combined with the levels of the substrate factor achieving the best average at$_{2b2}$ with 32.78 units, followed by$_{at\ 2b1}$ with 29.84 units and in last place the combination a0b1 with 17.39 units of roots. Factor B (substrates) presented statistical differences when combined with the levels of mycorrhizal factor, achieving the highest average $b_{2a2}$ with 32.78 units, followed by b1a2 with 29.84 units and b1a0 with 17.39 root units (Table 4).

Table 4 Tukey (0.05) in number of roots (unit), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | Unit | B | To | Unit |
| A1 | b0 | 23.06a | b2 | A0 | 20.78a |
| A2 | b0 | 21.78b | b0 | A0 | 19.17b |
| A0 | b0 | 19.17c | b1 | A0 | 17.39c |
| A2 | b1 | 29.84a | b2 | A1 | 27.06a |
| A1 | b1 | 25.55b | b1 | A1 | 25.55b |
| A0 | b1 | 17.39c | b0 | A1 | 23.06c |
| A2 | b2 | 32.78a | b2 | A2 | 32.78a |
| A1 | b2 | 27.06b | b1 | A2 | 29.84b |
| A0 | b3 | 20.78c | b0 | A2 | 21.78c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza

b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar ($p>0.05$)

Root dry matter.- Significant differences ($p<0.01$) were determined for root dry matter, in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When performing the ANOVA of the simple effects of the AxB interaction, significant differences ($p<0.01$) in factor A were determined with respect to the levels of factor B (b0, b1 and b2). Likewise, for factor B at each level of factor A (a1 and a2), for level a0 no significant differences were determined ($p>0.05$.). With Tukey (0.05) the mycorrhizal factor presented statistical differences when combined with the levels of the substrate factor, reaching an average of 2b2 with 36.01%, at1b2 with 30.96% and a0b0 with 13.10%. Likewise, for factor B (substrates) there were statistical differences when combined with factor A levels (mycorrhizae), where b2a2 obtained 36.01%, b2a1 30.96% and b0a0 achieved 13.10% (Table 5).

Table 5 Tukey (0.05) in root dry matter (%), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | cm | B | To | cm |
| A2 | b0 | 22.11a | b2 | A0 | 15.20a |
| A1 | b0 | 18.36b | b1 | A0 | 14.83b |
| A0 | b0 | 13.10c | b0 | A0 | 13.10c |
| A2 | b1 | 28.47a | b2 | A1 | 30.96a |
| A1 | b1 | 23.67b | b1 | A1 | 23.67b |
| A0 | b1 | 14.83c | b0 | A1 | 18.36c |
| A2 | b2 | 36.01a | b2 | A2 | 36.01a |
| A1 | b2 | 30.96b | b1 | A2 | 28.47b |
| A0 | b3 | 15.20c | b0 | A2 | 22.11c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar (p>0.05)

Seedling height.- According to ANOVA, significant differences (p<0.01) were determined in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When prioritizing the simple effects of the AxB interaction in the ANOVA (Gabriel, 2021), significant differences (p<0.01) were found in factor A with respect to the levels of factor B (b0, b1, and b2). Likewise, for factor B at each level of factor A (a0, a1 and a2). When performing the Tukey test (0.05), factor A (mycorrhizae) presents statistical differences when combined with substrate levels, with the highest average reaching the a2b2 combination with 16.44 cm followed by $a_{2b1}$ with 15.99 cm and the a0b0 combination reaching the lowest average with 9.20 cm in height. As for factor B (substrates), there are statistical differences when combined with the levels of mycorrhizae factor, with the highest average being the combination b2a2 with 16.44 cm, followed by b1a2 with 15.99 cm and achieving the minimum average $b_{0a0}$ with 9.20 cm of seedling height (Table 6).

Table 6 Tukey (0.05) in seedling height (cm), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | cm | B | To | cm |
| A1 | b0 | 13.17a | b2 | A0 | 11.16a |
| A2 | b0 | 11.84b | b1 | A0 | 9.66b |
| A0 | b0 | 9.20c | b0 | A0 | 9.20c |
| A2 | b1 | 15.99a | b2 | A1 | 15.84a |
| A1 | b1 | 14.45b | b1 | A1 | 14.45b |
| A0 | b1 | 9.66c | b0 | A1 | 13.17c |
| A2 | b2 | 16.44a | b2 | A2 | 16.44a |
| A1 | b2 | 15.84b | b1 | A2 | 15.99b |
| A0 | b3 | 11.16c | b0 | A2 | 11.84c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza

b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar (p>0.05)

Number of leaves.- When analyzing the data with the ANOVA, significant differences (p<0.01) were determined in the sources of variation of factor A (mycorrhizae), B (substrates) and in the AxB interaction. When performing the single-effect ANOVA of AxB, significant differences (p<0.01) in factor A were determined with respect to the levels of factor B (b0, b1 and b2). Likewise, for factor B at each level of factor A (a1 and a2), however, for level a0 no significant differences were determined (p>0.05). With Tukey (0.05) for the number of leaves, significant differences were observed for mycorrhizae when mixed with the substrate component, with the highest average being found in a2b2 with 164.39 units, followed by $a_{1b2}$ with 157.56 units and obtaining the lowest average a0b0 with 101.72 units of leaves. As for factor B (substrates), it presents statistical differences when combined with the levels of the mycorrhizal factor , achieving the highest average b2a1 with 164.39 units, followed by b2a2 with 157.56 units and in last place it was $b_{0a0}$ with 101.72 units of leaves (Table 7).

Table 7 Tukey (0.05) in number of sheets (unit), for the simple effects of factor A at each level of factor B; and, the simple effects of factor B at each level of factor A

| Factor | | Average | Factor | | Average |
|---|---|---|---|---|---|
| To | B | Unit | B | To | Unit |
| A1 | b0 | 138.28a | b2 | A0 | 106.83a |
| A2 | b0 | 125.78b | b1 | A0 | 105.67b |
| A0 | b0 | 101.72c | b0 | A0 | 101.72c |
| A2 | b1 | 156.56a | b2 | A1 | 164.39a |
| A1 | b1 | 144.33b | b1 | A1 | 144.33b |
| A0 | b1 | 105.67c | b0 | A1 | 138.28c |
| A2 | b2 | 164.39a | b2 | A2 | 157.56a |
| A1 | b2 | 157.56b | b1 | A2 | 156.56b |
| A0 | b3 | 106.83c | b0 | A2 | 125.78c |

A: Mycorrhizae, B: Substrates

a0: No mycorrhiza, a1: Native mycorrhiza, a2: Industrial mycorrhiza

b0: Peat (50%) + agricultural land (33.33%) + sand (16.67%), b1: Peat moss (50%) + sand (25%)+humus (25%), b2: Mixture 60% (Peat + agricultural land + sand)+ humus 40%

Equal letters within columns are statistically similar ($p>0.05$)

## 4. Discussion

Pine seedlings inoculated with the fungus ( Boletus edulis) have greater root development, which achieves better nutrition and this influences the growth of seedling height, number of leaves, stem diameter, percentage of attachment, greater number of roots and root length; likewise, the substrates fulfill the function of supporting the plant and also serving as a reserve of nutrients, which can be natural or synthetic, of organic or mineral origin, pure or also blended.

Survival.- With industrial mycorrhiza and 60% substrate (peat + agricultural land + sand) + 40% humus (a2b2 treatment), an average of 98% of engraftment was achieved. When compared to what was obtained by Bobadilla et al., (2018), who worked with substrates enriched with 40% humus, Pinus radiata achieved 100% of engraftment. Escamilla et al. (2020), in fresh pine sawdust substrates with composted pine bark and controlled-release fertilization, achieved more than 90% rooting in Pinus patula cuttings. Vergara (2004) reported a 93% survival rate in transplanting Pinus radiata plants inoculated with mycorrhizal moss. Ancco (2019), obtained 98.08% of engraftment with commercial mycorrhizae (Boletus edulis) in the propagation of Pinus radiata in sand and peat substrate. The failure of conifer reforestation projects in different countries has been attributed to the absence of mycorrhizal fungi (Vozzo, l984, cited by Vergara, 2004; Gómez, 2018; Ancco, 2019).

Root system. - With industrial mycorrhiza and 60% substrate (peat + agricultural land + sand) + 40% humus (a2b2 treatment), an average of 31.45 cm of root length was obtained; however, Bobadilla et al., (2018), in substrates enriched with 40% humus, in Pinus radiata It achieved a root length of 15.50 cm at 120 days. The development of mycorrhizae on pine roots is in loose-textured (sandy) soils with a high content of decomposed organic matter (humus) and good aeration (Guido, 1984, cited by Ancco, 2019; Bobadilla et al., 2018).

Also, with the a2b2 treatment, an average of 32.78 root units were achieved; on the other hand, Bobadilla et al., (2018), achieved 11.83 root units at 120 days. Ancco (2019), obtained an average of 13.50 roots. The number of short roots is almost double in infected plants compared to non-infected ones, and the presence of mycorrhizal fungi delays the absorption or loss of short roots (FAO and Pronamachs, 2017). Likewise, in dry matter, the highest percentage was obtained (36.01%), compared to Bobadilla et al., (2018) who achieved 29.95% of root dry matter at 120 days. Ancco (2019), obtained an average of 6.75 g of dry weight per seedling. Mycorrhizal fungi (Boletus edulis) have a positive effect on growth and vigor, as well as tolerance and/or resistance to biotic and abiotic agents present in the environment where seedlings develop (Gómez 2018; Quiroz-Mojica et al., 2021; Tafur et al., 2022). Limache (1985) cited by Vergara (2004), indicates that in order to produce and satisfactorily guarantee the development of pine seedlings, attention must be paid to the mycorrhizal process. Mycorrhizal roots, by occupying a greater volume of the soil, allow them to compete with greater advantage for soil nutrients (FAO and Pronamachs, 1998; Ancco, 2019), in addition to increasing its useful life (Vergara, 2004). Arbuscular mycorrhizal fungi native to soils with different uses exhibit low potential to develop mycorrhizal symbiosis (Martínez et al., 2019).

Seedling characteristics.- Similarly, with industrial mycorrhiza and 60% substrate (Peat + agricultural land + sand) + 40% humus (a2b2 treatment), the highest seedling height was obtained with 16.44 cm; however, Bobadilla et al., (2018), in substrates enriched with 40% humus, in Pinus radiata achieved a height of 12.17 cm at 120 days. Luna (2019), with perlite substrate (50%), sand (50%) and fertilization, obtained 18.79 cm in height at 180 days in Pinus taeda. Trevizan and Aguilar (2020), in queñoa (Polylepis rugulosa) reached a height of 12.8 cm per year, using 33% soil from the sector + 33% compost + 33% peat + 1% sand. Ancco (2019), obtained a height of 45.97 cm, with commercial mycorrhizae (Boletus edulis) in the propagation of Pinus radiata in sand and peat substrate, at nine months. The mycorrhizal association is one of the factors that contribute to the mineral nutrition, growth and development of the genus Pinus and other forest species (Vergara, 2004). According to Mexal and Landis (1990), plant height is a good predictor of future height in the field. Increased soil volume, thanks to the large number of fine hyphae of arbuscular mycorrhizal fungi, appears to be largely responsible for the considerable differences in survival, growth rate, and yields between mycorrhizal and non-mycorrhizal plants, particularly in degraded soils (Habte et al., 1987).

Also with the a2b2 treatment, the highest number of leaves per seedling was achieved (164.39), compared to Bobadilla et al., (2018) who obtained 138.20 units. Mycorrhizal association is one of the factors contributing to the growth and development of the genus Pinus and other forest species and is reflected in the size, thickness of the plant, robustness, coloration, formation of needles (pine leaves) and a more abundant root system (Bobadilla et al., 2018; Gómez, 2018; Álvarez-Manjarrez et al., 2021; Carrillo-Saucedo et al., 2022).

## 5. Conclusions

It was determined, at 120 days in nursery conditions, that in the interaction of the AxB factors (mycorrhizae with substrates), the combination of the levels $at_{2b2}$ (industrial mycorrhizae, with the mixture of [peat + agricultural land + sand] + humus), significant results were obtained ($p<0.01$). The percentage of attachment was 98.00%, seedling height 16.44 cm, number of leaves 164.39 units, root length 31.45 cm, number of roots 31.45 units and percentage of root dry matter 36.01%. The results obtained demonstrate the importance of mycorrhizae in the propagation of pine seedlings for afforestation and reforestation purposes, aimed at mitigating climate change, promoting the sustainable use of natural resources and improving the well-being of Andean communities.

Contributions from authors

Conceptualization: Bedoya-Justo E, Quispe R. Data curation: Bedoya-Justo E, Quispe R. Formal analysis: Bedoya-Justo E, Quispe R. Acquisition of funds: Bedoya-Justo E, Quispe R. Research: Quispe R, Bedoya-Justo E, Ruiz W, Chaparro E, Cuentas O. Methodology: Bedoya-Justo E, Quispe R. Project management: Bedoya-Justo E, Quispe R. Resources: Quispe R, Supervision: Quispe R. Writing of the original draft: Bedoya-Justo E, Quispe R, Ruiz W, Chaparro E, Cuentas O. Writing, revision and editing: Bedoya-Justo E, Quispe R, Ruiz W, Chaparro E, Cuentas O.

## WORKS CITED

Ancco Y. 2019. Evaluation of the mycorrhizal inoculum of the fungus (Boletus edulis) in the production of pine seedlings (Pinus radiata D. Don) in Andahuaylas. Undergraduate thesis, Universidad Nacional de San Antonio Abad del Cusco, Peru. https://repositorio.unsaac.edu.pe/handle/20.500.12918/5017

Álvarez-Manjarrez J, Solís A, Villarruel-Ordaz J, Ortega-Larrocea M, Garibay-Orijel R. 2021. Mycorrhizae of the tropical deciduous forest and other fungal symbioses. Act. Bot. Mex. (128): e1906. https://doi.org/10.21829/abm128.2021.1906

Arteaga M, Tafur S, Pérez G, Pastor S, Batista A. 2020. Characterization of mycorrhizal colonization in Retrophyllum rospigliossi Pilger in the Huamantanga forest, Peru. Cuban Journal of Forest Sciences, 8(3): 535-549. cfores.upr.edu.cu/index.php/cfores/article/view/653

Bobadilla L, Huarhua T, Bedoya-Justo E. 2018. Application of nursery production technology for two varieties of pine in the district of Santo Tomás, province of Chumbivilcas, Cusco region. Revista Ciencia y Tecnología para el desarrollo-UJCM, 4(7): 29-33. https://revistas.ujcm.edu.pe/index.php/rctd/article/view/99/83

Carrillo-Saucedo S, Puente-Rivera J, Montes-Recinas S, Cruz-Ortega R. 2022. Mycorrhizae as a tool for ecological restoration. Act. Bot. Mex., (129): e1932. https://doi.org/10.21829/abm129.2022.1932

Cuellar J, Salazar E, Fernández K. 2015. Effect of tropical forest use change on total carbon stored, Aguaytía basin, Peru. Revista Forestal del Perú, 27(1-2): 7-26. repositorio.inia.gob.pe/handle/inia/559

Cuellar J, Salazar E. 2016, October. Dynamics of carbon stored in the different land use systems of Peru; basis for a climate change mitigation strategy. XII National Forestry Congress - CONAFOR. Lima, Peru. https://alicia.concytec.gob.pe/vufind/Record/INIA_4cd231be4b5d74dce6da77028c4e3436

Escamilla N, Aldrete A, Vargas J, Villegas A, López M. 2020. Vegetative propagation of Pinus patula Schiede ex Schltdl. et Cham. in different substrates. Revista Fitotecnia Mexicana, 43(2): 215-221. https://doi.org/10.35196/rfm.2020.2.215

Fernández-Golfín, J. 1996. User manual Insignis pine from the Basque Country. Technical Wood Centre, Vitoria. Agrobyte, Spain,
Gabriel J, Valverde A, Indacochea B, Castro C, Vera M, Alcivar J, Vera R. 2021. Experimental Designs: Theory and Practice for Agricultural Experiments (2nd edition). State University of the South of Manabí, Guayaquil, Ecuador. http://142.93.18.15:8080/jspui/handle/123456789/116
Gómez M. 2018. Growth of pine seedlings (Pinus radiata) under the action of mycorrhizal fungal extract (Boletus edulis) under nursery conditions Chuquibambilla - Grau - Apurimac. Undergraduate thesis, Universidad Nacional Micaela Bastidas de Apurímac, Peru. https://repositorio.unamba.edu.pe/handle/UNAMBA/616
Habte M, Fox R, Huang R. 1987. Determination of the efficacy of vesicular-arbuscular mycorrhizae by monitoring the P status of the subaflets of an indicator plant. Communications in Soil Science and Plant Analysis, 18(2): 1403-1420. https://doi.org/10.1080/00103628709367907
National Institute of Natural Resources (INRENA). 2005. National Reforestation Plan. Ministry of Agriculture - MINAG. Lima, Peru. https://www.agrorural.gob.pe/dmdocuments/bnsf/plan_nacional_de_reforestacion.pdf
Lozano-Povis A, Alvarez-Montalván C, Moggiano N. 2021. Climate change in the Andes and its impact on agriculture: a systematic review. Scientia Agropecuaria, 12(1): 101-108. http://dx.doi.org/10.17268/sci.agropecu.2021.012
Luna C. 2019. Evaluation of substrates and fertilizer concentrations on the growth of tadea pine (Pinus taeda L.) in nursery. Revista Agronómica del Noroeste Argentino, 39(1): 19-29. https://ri.conicet.gov.ar/handle/11336/108601
Martínez J, Osorio N, Garrido J. 2019. Effectiveness of native mycorrhizal-arbuscular fungi in soils with different agricultural uses. MVZ Córdoba Magazine, 24(2): 7256-7261. https://doi.org/10.21897/rmvz.1703
Mexal J, Landis T. 1990. Target seedling concepts: height and diameter. In: Symposium on Target Seedlings: Proceedings, Combined Meeting of Western Forest Nursery Associations. Forest Service, United States. https://rngr.net/publications/proceedings/1990/mexal.pdf
Ministry of the Environment (MINAM). 2011. The Peru of the forests. Super Gráfica EIRL, Lima, Peru. https://sinia.minam.gob.pe/documentos/peru-bosques
Moreira-Rivas E, Díaz-Lezcano M. 2022. Association of Phlebopus sp. with forest species of the urban trees of Asunción, Paraguay. Rev. cubana ciencias forestales. 10 (2): 197-214. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2310-34692022000200197&lang=es
Núñez, L. 2022. Reforestation of dry forest for the prevention of river overflows in Peru: the case of the district of Íllimo, province and region Lambayeque (2018-2019). Kawsaypacha Journal: Society and the Environment. (9): 108-123. http://dx.doi.org/10.18800/kawsaypacha.202201.005
Oliva M, Vacalla F, Pérez D, Tucto A. 2014. Forest nursery for the production of seedlings of native forest species: experience in Molinopampa, Amazonas - Peru. Peruvian Amazon Research Institute - IIAP, Amazonas, Peru. https://repositorio.iiap.gob.pe/handle/20.500.12921/348
Food and Agriculture Organization of the United Nations (FAO), National Program for Watershed Management and Soil Conservation (Pronamachs). 1998. Phytosanitary and mycorrhizal aspects in forest nurseries in the Peruvian highlands 1996-1997. FAO and Pronamachs, Lima, Peru. https://catalogobiam.minam.gob.pe/cgi-bin/koha/opac-detail.pl?biblionumber=1919
Food and Agriculture Organization of the United Nations (FAO), National Forest and Wildlife Service (SERFOR). 2017. Our forests in numbers. National Forest and Wildlife Inventory. Communication and Sustainability SAC, Lima, Peru. https://cdn.www.gob.pe/uploads/document/file/1269105/Nuestros%20Bosques%20en%20N%C3%BAmeros.pdf
Pérez-Luna Y, Álvarez-Gutiérrez P, Mendoza D, Mendez-Trujillo V. 2019. Evaluation of the presence of arbuscular mycorrhizal fungi in a pine-oak forest in Chiapas, Mexico. IDESIA (Chile) 37(1): 67-73. http://dx.doi.org/10.4067/S0718-34292019005000401
Quintero-Gradilla S, Jardel-Peláez E, Cuevas-Guzmán R, García-Oliva F, Martínez-Yrizar A. 2020. Post-fire change in the structure and composition of the tree stratum and fuel load in a Pinus douglasiana forest in Mexico. Wood and forests. 25 (3): e2531888. https://doi.org/10.21829/myb.2019.2531888

Quiroz-Mojica L, Daza-Mendoza M, Díaz-Muegue L, Melo-Rios A, Peñuela-Mesa G. 2021. Effect of biochar, arbuscular mycorrhizae and Guazuma ulmifolia, in the rehabilitation of mining soils. Terra Latinoamericana. 39: e709. https://doi.org/10.28940/terra.v39i0.709
Romero-Arenas O, Flores A, Rivera J, Hernández F, Conrado J, Villa N, Landeta G. 2019. Production of Pinus pseudostrobus seedlings in compost based on Shiitake waste in a nursery. Madera y Bosques, 25(1): e2511675. https://doi.org/10.21829/myb.2019.2511675
Sánchez F. 2013. Influence of active substrates for the growth of pine (Pinus radiata Don) produced under forest nursery conditions in the community of Cuticsa - Santo Tomas de Pata - Angaraes - Huancavelica. Undergraduate thesis , National University of Huancavelica, Peru. https://repositorio.unh.edu.pe/items/1703ca1f-2e5c-47ce-a8d3-2ebad6ab092e
National Meteorology and Hydrology Service of Peru (SENAMHI). 2018. Meteorological bulletins. SENAMHI, Cuzco, Peru. https://www.senamhi.gob.pe/?p=boletines
Tafur E, Veneros J, García L, Gamarra O, Farje J, Santistevan M. 2022. Non-destructive techniques for estimating aboveground forest biomass. IDESIA (Chile). 40 (3): 7-17. http://dx.doi.org/10.4067/S0718-34292022000300007
Trevizan J, Aguilar R. 2019. Evaluation of the nursery process to obtain queñoa (Polylepis rugulosa Bitter) plants with different types of substrates in the town of Chapiquiña, XV region of Arica and Parinacota, Chile. IDESIA (Chile), 37(3): 89-97. http://dx.doi.org/10.4067/S0718-34292019000300089
Vallejo M, Cob J. 2021. Topics and perspectives of scientific research on the genetic improvement of perennial species at INIFAP. Mexican Journal of Forest Sciences. 12 (SPE): 154-176. https://doi.org/10.29298/rmcf.v12iespecial-1.1089
Vergara K. 2004. Response of the mycorrhizal inoculum of the fungus Scleroderma verrucosum in the production of Pinus radiata D. Don seedlings in Jauja, Junin. Undergraduatethesis, Universidad Nacional Agraria La Molina, Lima, Peru. https://repositorio.lamolina.edu.pe/handle/20.500.12996/1741
Zanabria R, Cuellar J. 2015. Total carbon stored in the deposits of different land use systems of the high Andean ecosystem, Mantaro Valley, Junín. Xylem, 28(1): 43-52. http://dx.doi.org/10.21704/x.v28i1.597