# NATIVE MYCORRHIZAE FROM ETHIOPIA IMPROVE TREE GROWTH AND SEEDLINGS SURVIVAL CONTRIBUTING TO THE GREEN LEGACY PROGRAM †

# [MICORRIZAS NATIVAS DE ETIOPÍA MEJORAN EL CRECIMIENTO DE ÁRBOLES Y LA SUPERVIVENCIA DE LAS PLÁNTULAS CONTRIBUYENDO AL PROGRAMA LEGADO VERDE]

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## SUMMARY

**Background**: A rapid production of tree seedlings in nurseries with a high survival rate after transplanting is important to respond to the current demand for programs of restoration of arid environments by forestation. The low level of seedlings' survival and establishment, caused by low moisture and nutrient content of soils, has been a bottleneck to reaching the target of the forest national restoration in Ethiopia of last years. It is suggested that, inoculation with root-associated plant growth promoting microorganisms could help to ameliorate this scenario and also respond to the Ethiopia's Green Legacy Program. **Objective**: To assess the potential of inoculation with arbuscular mycorrhizal fungi (AMF) native to Ethiopia to improve the survival and growth of trees that could be used in afforestation programs in Ethiopia. **Methodology**: The study was carried out in three stages: (1) soil samples associated with roots of selected acacia species ($T_1$-AMF of *A. abyssinica*, $T_2$-AMF of *A. seyal*, $T_3$-AMF of *A. tortilis* and $T_4$-Control) were collected of highland and lowland areas from Ethiopia, (2) Sorghum (*Sorghum bicolor* (L.), provided by the Melkasa Agricultural Research Center-(MARC) served as a trap plant for the AMF consortium multiplication and (3) plant growth promotion by AMF was assessed throughout inoculations of seedlings of *Delonix regia* (Hook.) Raf., *Sesbania grandiflora* (L.), *Cassia fistula* L., and *Azadirachta indica* A. Juss., trees. **Results**: All inoculated seedlings showed significantly greater responses in all growth and mycorrhizal parameters over the non-inoculated trees. Consortium $T_2$-AMF associated to roots of *A. seyal* from lowlands of Batu, showed significantly greater responses in all plant growth and mycorrhizal parameters over the AMF inoculums associated to other tree species evaluated. Significant and positive correlations were found between mycorrhizae and plant-growth parameters. **Implications:** Our results suggest that inoculation with native arbuscular mycorrhizal fungi indigenous from Ethiopia has the potential to significantly enhance survival and growth rates of tree seedlings. This could thereby advance national reforestation goals and addressing challenges in seedling establishment in arid environments. **Conclusion**: The potential for growth promotion and establishment of tree seedlings evidenced, implies that further efforts should be directed towards the in-mass production of AMF-based inoculants, particularly associated with *A. seyal* roots.
**Keywords:** Acacia trees; mycorrhizal fungi; forestation program; inoculation; seedlings

## RESUMEN

**Antecedentes**: Una producción rápida de plántulas de árboles en viveros con una alta tasa de supervivencia tras el trasplante es importante para responder a la demanda actual de programas de restauración de ambientes áridos por forestación. El bajo nivel de supervivencia y establecimiento de las plántulas, causado por el bajo contenido de humedad y nutrientes de los suelos, ha sido el factor limitante para alcanzar el objetivo de la restauración forestal nacional en Etiopía de los últimos años. Se sugiere que la inoculación con microorganismos promotores

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de crecimiento vegetal asociados a las raíces de las plantas, podría contribuir a mejorar esta situación y también responder al Programa Legado Verde de Etiopía. **Objetivo**: Evaluar el potencial de la inoculación con hongos micorrícicos arbusculares (AMF) nativos de Etiopía, para mejorar la supervivencia y el crecimiento de plántulas que podrían ser utilizadas en programas de forestación en Etiopía. **Metodología**: El estudio se llevó a cabo en tres etapas: (1) colecta de muestras de suelo asociadas a raíces de acacia ($T_1$-AMF de *A. abyssinica*, $T_2$-AMF de *A. seyal*, $T_3$-AMF de *A. tortilis* y $T_4$-Control) de zonas de tierras altas y bajas de Etiopia, (2) multiplicación de consorcios de AMF nativos en raíces de sorgo (*Sorghum bicolor* L.), proporcionado por el Centro de Investigación Agrícola de Melkasa (MARC), como planta trampa, y (3) evaluación de la promoción del crecimiento en plántulas de *Delonix regia* (Hook.) Raf, *Sesbania grandiflora* (L.), *Cassia fistula* L. y *Azadirachta indica* A. Juss., por la inoculación con los consorcios con AMF. **Resultados**: Todas las plántulas inoculadas mostraron respuestas significativamente mayores en todos los parámetros de crecimiento y micorrización que las no inoculadas. El consorcio $T_2$-AMF asociado a raíces de *A. seyal* autóctono de tierras bajas de Batu, mostró respuestas significativamente mayores en todos los parámetros de crecimiento y micorrización de las plantas, que los inóculos asociados a otras especies arbóreas evaluadas. Se encontraron correlaciones significativas y positivas entre los parámetros de micorrización y crecimiento de las plantas. **Implicaciones:** Nuestros resultados sugieren que la inoculación con hongos micorrícicos arbusculares autóctonos de Etiopía tiene el potencial de mejorar significativamente las tasas de supervivencia y crecimiento de las plántulas de árboles. De este modo se podría avanzar en los objetivos nacionales de reforestación, y hacer frente a los retos que plantea el establecimiento de plántulas en entornos áridos. **Conclusiones**: El potencial para la promoción del crecimiento y el establecimiento de plántulas de árboles evidenciado, implica que los futuros esfuerzos se deben dirigir hacia la producción en masa de inoculantes basados en AMF, particularmente asociados a las raíces de *A. seyal.*
**Palabras clave:** Acacia; hongos micorrícicos; programa de forestación; inoculación; establecimiento de plántulas.

## INTRODUCTION

The Government of Ethiopia has been working to rehabilitate degraded lands and restore forests through national tree planting campaigns since this millennium. These development activities have been accomplished under the country's National Green Development program, to reduce climate change and environmental degradation. The Ethiopian government announced a larger goal of planting 20 billion trees during a four-year period (2019-2023). However, according to reports of Adama Woreda Agriculture and Natural Resources Office (AWANRO) during 2020 and 2021, the survival rate of seedlings was lower than expected and ranged from 40-50% respectively (AWANRO, 2021). So that, the original projection is not being achieved. In order to meet the objective of promoting the contribution of forestry to agriculture, water and energy, it is proposed to cover 22 million hectares of degraded land in Ethiopia with forests by 2030 (Kassa, 2018; Mekonnen, 2018).

The low level of seedlings' survival and establishment in Ethiopia's fields have been a bottleneck for the achievement of the objectives of the national restoration target (Eba, 2017; Asmelash *et al*., 2019). In fact, it caused loss of a huge amount of money allocated for this purpose. Poor survival and establishment of trees after transplanting in fields were caused by low moisture and nutrient content of soils (Mahari, 2014; Asmelash *et al*., 2019). In addition, the selection of suitable trees for each particular site, providing the necessary aftercare and the use of relevant technologies to improve the moisture and nutrient balance of the seedlings should have been considered. Since some soil micro-organisms can contribute to the mineral nutrition of plants and to the improvement of soil quality, biological inoculation could be a promising alternative. One of available alternative could be the inoculation of tree seedlings with arbuscular mycorrhizal fungi (AMF) (Asmelash *et al*., 2016), which are considered as natural growth regulators of a majority of terrestrial flora. The AMF establish a mutualistic symbiotic association with roots of their host plants and, as results of mycorrhizae formation, increases in water and nutrient uptake are usually detected both under nursery and field conditions (Diagne *et al*., 2020) that are mediated by increases in the volume of soil explored by the root system and external AMF mycelium (dos Santos *et al*., 2017). They also confer the plant's resistance against roots pathogens and improvement in water relations (Poveda *et al*., 2020). In addition to the benefits on host plants, ecosystem services on the soil by the formation of mycorrhizae have been detected. For example, higher stable aggregate content in soils with higher AMF presence has been detected and associated with the release of binding glycoproteins known as glomalins (Smith and Read, 2008). In addition, the contribution of soil macroaggregate trapping by the external mycelium of AMFs has been evidenced (Syamsiyah *et al*., 2018). Therefore, the use of inoculation with AMF to improve crop performance and soil quality is advised (Barrow, 2012; Kuila and Ghosh, 2022).

Tran *et al*., (2019) examined the growth and nutritional responses of agriculturally important plant species, with and without inoculation with AMF, and found that mycorrhizal colonization of roots, plant growth, and plant nutrient responses was increased after inoculation. Ortega *et al*., (2004) reported that at afforestation sites devoid of mycorrhizal propagules, seedlings never develop

fully and often die. Thus, the selection and inoculation of mycorrhizal fungi with beneficial functions can contribute to the establishment and plantlet survival by enhancing nutrient and water acquisition and also by increasing tolerance to transplant stress (dos Santos *et al*., 2017). In spite of their potential and benefits, the large-scale use of AMFs is still limited, mainly due to the lack of availability of inoculant in high quantities, at low cost and high quality (IJdo *et al*., 2011). In addition, studies on the role of AMF in rehabilitation and regeneration of degraded lands are very limited or nil. Therefore, in view of the urgent need based on low seedling growth and survival, and also in response to national revegetation programs, a promising alternative should take into consideration the inoculation with indigenous AMF. The first step is then to select the inoculum (AMF consortium) that could be most efficient for the survival and establishment of trees considered in the reforestation plan. Subsequent confirmation of their potential in the field, will contribute to reduce costs of chemical fertilizers and to produce seedlings with good vigor which would translate into high survival and growth at the field.

Therefore, aimed to answer the goal of the Green Legacy Program of Ethiopia, the objective of this study was to evaluate the potential of native AMF inoculums isolated from highland and lowland areas, to improve the growth and survival of tree seedlings.

## MATERIALS AND METHODS

### Description of the study area

The study was performed in selected lowland areas of central Rift Valley and highland regions of Ethiopia. Selection of areas was based on prior studies of AMF diversity and abundance associated with acacia trees under different land use systems (Belay *et al.*, 2013). The Central Rift Valley is a lowland area of Ethiopia, in which Batu and Bishoftu have woody grassland where naturally different acacia tree species are dominant. The high land area is Sululta, the tertiary of Addis Ababa. Bishoftu and Batu are located in the East Showa zone, Oromia Regional State, at an altitude of 1600 to 1960 above sea level. The zone extends between 7º33’N-9º08’N and from 38º24’E- 40º05’E, with a total area coverage of approximately 13765.7 km$^2$. The annual rainfall distribution ranges from 600 mm to 1000 mm with average of 816 mm, with the temperature range from 10ºC in the uplands to over 30ºC in the depressions of the Rift Valley with a mean temperature of 20 ºC (CSA, 2020/21). Sululta is located in the central part of Ethiopia, in the Oromia Special zone, 23 km from Addis Ababa to the north. Geographically, the study area extends from 9º30'00"N to 9º12'15"N latitude and 38º42'0"E to 38º46'45" E longitude with the altitude of 2589 m above sea level. The administrative area of the town is about 4471 ha. Sululta has similar climatological characteristics to Addis Ababa. Globally, it is a part of a tropical humid climatic region, which is characterized by warm temperatures and high rainfall (the maximum annual rainfall is 1447 mm with a mean of 1140 mm and a minimum of 834 mm). The soils of the area are derived from Mesozoic sedimentary and volcanic rocks. The major soil types of the Sululta area are Chromic Luvisols (Gelan, 2021).

### Soil sampling

Soil samples were collected in February 2021. Triplicate samples were taken randomly from each site within a 100 m$^2$ (10m x 10m) quadrant. Soil associated with the roots of *Acacia abyssinica* from Sululta (the high land area) and of *A. seyal* and *A. tortilis* from Batu and Bishoftu (the low land areas) was collected (Table 1). Within each site, three replicates of each acacia tree species were randomly selected, and about 3 kg of soil were taken (0-30 cm depth). After collection, samples were pooled into a composite sample (9 kg from each sample location for each species). As result, 45 kg of soil samples were collected in sterilized plastic bags and stored at room temperature until use.

### Establishment of trap cultures for AMF inoculum production

In order to obtain many healthy and infective AMF propagules for inoculation, a trap culture was established in the greenhouse at Adama Science and Technology University (ASTU) for four months (March to June 2021), following the protocol of INVAM (http://invam.caf.wvu.edu). Trap cultures were set up in triplicates for the five collected soil samples (*A. seyal* and *A. tortilis* each from Bishoftu and Batu and *A. abyssinica* from Sululta). According to Belay *et al.* (2013), the dominant AMF species associated with these acacia trees based on the relative abundance and frequency of spores were: *Claroideoglomus claroideum, Claroideoglomus etunicatum, Claroideoglomus luteum, Funneliformis geosporus,* and *Glomus aggregatum.*

To set up trap cultures, each collected soil sample was thoroughly mixed (1:1 v/v) with washed and autoclaved (121 ºC, 1 hour, twice with intervals of 24 h) sand, and about 3 kg of each mixed substrate was transferred to 100 cm$^3$ plastic pots that were irrigated for three days prior to seeding. Seeds (80/pot) of sorghum [*Sorghum bicolor* (L.), Melkam cv.], selected as trap plants for its mycotrophic capacity and to induce high spore multiplication (INVAM, http://invam.caf.wvu.edu), were provided by the Melkasa Agricultural Research Center (MARC). Seeds were surface sterilized by soaking

for 15 min in a 0.5 % sodium hypochlorite solution and washing with sterile water. Seeds were sown at a 2 cm depth in each plastic pot and covered with sterilized sand. All seedlings were grown in the greenhouse under natural light and temperature conditions during four months. Plants were irrigated daily as needed, and no fertilizer was applied. To induce spore production, watering was reduced during the last week of the trap cultures growth. At the end of trap cultures growth, aerial part of trap plants were cut near the base, and roots, substrate and accompanying microflora were air-dried and stored in zipped plastic bags at room temperature for 30 days before inoculation (INVAM, http://invam.caf.wvu.edu).

**Assessment of root colonization and spore density of trap culture**

Roots of seedlings of each trap culture were washed several times in tap water and cut into segments of about 1–2 cm long. To confirm the quality of each inoculum, mycorrhizal colonization on the roots of the trap plants and spore density in the substrate were quantified. Briefly, about 0.5 g of root segments were cleared in 10 % (w/v) KOH at 90 ºC for 1 hour in water bath. Roots were further bleached with alkaline hydrogen peroxide (10 % $H_2O_2$) for 3 min at room temperature. Thereafter, the roots were treated with 2 % HCl (v/v) for 15-20 min at room temperature and finally stained in 0.05 % w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90 °C for one hour in water bath (Brundrett *et al*., 1996). Samples were washed thoroughly with distilled water at the end of every step except HCl treatment. The samples were left in distaining solution (lactoglycerol) for more than two days in a dark room. Finally, roots were mounted on microscopic slides and covered with 24×24 mm coverslips. AMF colonization was assessed according to the method of Mc Gonigle *et al.* (1990). A total of 150 intersections were taken for each subsample to estimate percent AM root colonization under a compound microscope at a magnification of 100X. The presence of arbuscular colonization (AC) and vesicular colonization (VC) were calculated by dividing the count for the 'arbuscules' and 'vesicles' categories, respectively by the total number of intersections. Total colonization (TC) was calculated a proportion of non-negative intersections. The assessment of AMF spore density was performed by wet sieving and decanting method (Gerdemann and Nicolson, 1963), followed by centrifugation in water and in a 50 % sucrose solution (Brundrett *et al*., 1996). Spore density (SD) was quantified according to INVAM (http://invam.caf.wvu.edu).

**Experimental design and inoculation of AMF on selected trees**

In order to assess the efficiency of each inoculum in poor fertile soil, a soil with low pH and low phosphorus levels was collected from surrounding of Holeta Town (Ethiopia). The physical and chemical characteristics of the test soil were as reported by Belay and Assefa (2011), as described: P (6.44 ppm), N (1.66), organic matter content (1.549 %), pH (4.75), and EC (0.059 ds/m) . Plastic pots (capacity 3 kg, diameter of 15 cm, and 20 cm depth) were filled with 2.7 kg of the unsterilized poor fertile soil and 0.3 kg of each inoculum (where appropriate according to the inoculation treatments that will be described below).

For the growth promotion experiment, healthy selected seeds of *Azodichta indic*a (Neem), *Delonix regia*, *Sesbania grandiflora*, and *Cassia fistula* provided by Adama Woreda Agriculture and Natural Resources office, were surface disinfected (0.5 % sodium hypochlorite solution for 15 min, washing with sterilized water) and allowed to germinate on a 0.75 % (w/v) water agar for 48 h at 25 °C before planting or sowing. Then, two germinated seeds were sown at 2 cm depth in each plastic pot.

For each tree species, the following AMF inoculation treatments were established in a completely randomized design replicated three times: $T_1$-AMF of *A. abyssinica* from Sululta site, $T_2$-AMF of *A. seyal*, $T_3$-AMF of *A. tortilis* from Batu site and $T_4$-Control (Non-AMF inoculated, which received a mixture of the three inoculums in equal proportion, but sterilized). The AMF inocula for each experimental treatments (with respect to the site of origin) were selected based on the AMF spore density and percentage of the root colonization obtained during trap culture establishment (Table 1). The plants were watered 4 days a week and grown in a greenhouse (with natural light and temperature) for 4 months.

**Evaluation of growth promotion and mycorrhizal colonization**

The measurement of plant growth parameters was conducted twice at 60 and 120 days intervals after sowing. One seedling from each pot was uprooted at the end of 60 days and the remaining seedling from each pot was uprooted at the end of the growth season (120 days) for the measurement of growth parameters. At each harvest, shoot and root height (SH, RH, respectively) of plants, total leaves number (NL), root length (RL) fresh shoot mass (FSM) and fresh root mass (FRM) were recorded. After drying plant material at 70 $^0$C in an oven for 48 h, dry shoot and root mass (SDM and RDM, respectively) also were recorded. Roots were

processed to quantify mycorrhizal colonization as described above. Similarly, spore density in the substrate at 120 of plant growth (final harvest) was quantified as described above.

For each plant species and inoculation treatment, the mycorrhizal dependency (MD) were calculated as a percentage as follows (Plenchette, 1983):

$$\%\text{MD} = \frac{\text{DWM} - \text{DWNM}}{\text{DWM}} \times 100,$$

In which DWM represents dry weight of mycorrhizal (inoculated) plants and DWNM non-mycorrhizal (control) plants.

**Statistical analysis**

The statistical analysis of the data was performed using the SPSS software package (version 26.0). AMF spore density and percentage of root colonization and growth responses (plant shoot height, root height, number of leaves, dry and fresh weight of root and shoot) of the test plants data were subjected to one-way analysis of variance (ANOVA) using the treatments as factor. Tukey's honestly significant difference (HSD) post hoc test was used for pair-wise multiple mean comparisons tests. Pearson's correlation coefficients among parameters were analyzed. All the tests of statistical significance were decided at $p<0.05$.

## RESULTS

**AMF root colonization and spore density of the trap culture**

In order to obtain more infective and abundant AMF spores and propagules for inoculum production, the comparison of the inocula associated to roots of the same host plant but from different sampling locations (Batu and Bishoftu), and the establishment of trap culture under greenhouse conditions for multiplication, was required. Accordingly, five soil samples (associated to roots of *A. seyal* and *A. tortilis* each from Bishoftu and Batu and *A. abyssinica* from Sululta) were collected, AMF and accompanying microflora were multiplied and AMF root colonization and spore abundance were examined.

The density of AMF spores of the trap cultures ranged from 18.7 to 32.4 $g^{-1}$ (Table 1). There were significant differences in AMF spore density among the areas of sampling and the acacia species. Highest AMF spore density was found associated to roots of *A. seyal* from Batu and the lowest was recorded at the highland soil associated to the roots of *A. abyssinica*. All seedlings of sorghum in trap culture were colonized by AMF. The percentage of AMF root colonization significantly differed between the tree species in which multiplied soil was associated ($p\leq0.05$) (Table 1). Average of total colonization ranged between 56.9 % and 94.9 %. The highest percentage of root colonization of the trap plants was found in multiplied soil associated with *A. seyal* from lowland Batu, and the lowest colonization in the roots of multiplied soil from *A. abyssinica*.

**Growth response of the trees to AMF inocula *Azadirachta indica* A. Juss**.

At 60 and 120 days-growth, all plants inoculated with AMF showed significantly greater responses at all growth parameters over the non-inoculated control plants ($T_4$). Inoculation of *A. indica* (Neem) with AMF inocula of *A. abyssinica* ($T_1$) from Sululta and *A. seyal* ($T_2$) from Batu showed, in general, significantly greater responses in growth parameters in relation to those inoculated with AMF of *A. tortilis* ($T_3$) from Batu (Table 2). At 120 days-growth, plants inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater responses at all parameters over the $T_1$ and $T_3$ inoculated plants. Plants inoculated with AMF from *A. abyssinica* ($T_1$) and AMF from *A. tortilis* ($T_3$) showed comparable responses in SH, NL, FRM and DRM parameters at 120 days-growth.

***Delonix regia* (Hook.) Raf.**

At 60 and 120 days-growth, all plants inoculated with AMF showed significantly greater responses at all growth parameters over the non-inoculated control plants ($T_4$). After 60 days of growth, plants of *D. regia* inoculated with AMF of *A. seyal* ($T_2$) and plants inoculated with AMF of *A. abyssinica* ($T_1$) showed significant increases of all parameters over non-inoculated control plants (Table 3). However, plants inoculated with AMF from Batu's *A. seyal* ($T_2$) showed greater responses over the seedlings than inoculated with AMF of *A. abyssinica* ($T_1$) in RH, NL and FRM. After 120 days-growth, plants inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater responses over $T_1$ and $T_3$ inoculated plants.

***Sesbania grandiflora* (L.)**

At 60 days-growth, plants inoculated with AMF of all acacia species *A. abyssinica* ($T_1$) from Sululta, *A. seyal* ($T_2$) and *A. tortilis* ($T_3$) from Batu, showed significant greater responses in all growth parameters over the control ($T_4$), except in DSM (Table 4). Seedlings inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater growth responses in FSM and DRM than $T_1$ and $T_3$ inoculated plants. Similarly, seedlings inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater growth responses in RH and DRM than both $T_1$ and $T_3$ respectively.

At 120 days, the growth parameters showed variability among the treatments (Table 4). Accordingly, the results of all growth parameters of the seedlings inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater responses over seedlings that were the control ($T_4$) and other inoculated group ($T_1$ and $T_3$). Secondly, seedlings inoculated with AMF of *A. abyssinica* ($T_1$) and *A.*

**Table 1. Spore density and percentage of root colonization with arbuscules, vesicles and hyphae of arbuscular mycorrhizal fungi recorded at the trap cultures.**

| Sampling area | Source (host plant) of soil inoculum | SD ($g^{-1}$) | RC % | | |
|---|---|---|---|---|---|
| | | | AC % | VC % | TC % |
| Sululta | *A. abyssinica* | 19±0.4e | 25.2±0.0e | 14.6±0.1d | 56.9±0.2e |
| | *A. seyal* | 32±0.2a | 45.7±0.8a | 25.6±0.4a | 94.9±0.4a |
| | *A. tortilis* | 22±0.06d | 34.4±0.4c | 19.9±0.4c | 62.9±1d |
| | *A. seyal* | 28±0.1b | 41.7±0.4b | 27.3±0.4a | 79.1±1.1b |
| | *A. tortilis* | 22±0.1d | 28.4±0.4d | 22.5±0.4b | 61±0.5d |

Note- SD- spore density, RC- root colonization, AC- arbuscular colonization, VC – vesicular colonization, and TC-Total colonization. Different lowercase letters in the same column represent significant differences at 0.05 level; Mean values followed by the same letter are not significantly different at P< 0.05. Mean ± Standard error.

**Table 2. Effect of arbuscular mycorrhizal inoculation on growth characteristics of *Azadirachta indica*.**

| | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| 60 days-growth | SH (cm) | RH (cm) | NL | FSM (g) | FRM (g) | DSM (g) | DRM (g) |
| Treatment | | | | | | | |
| $T_1$ | 14.1±0.4a | 7.6±0.0b | 35±1a | 1.2±0.1b | 2.0±0.0a | 0.5±0b | 0.9±0a |
| $T_2$ | 14.5±0.2a | 7.8±0.4a | 29±1b | 1.3±0.0a | 1.7±0.0b | 0.7±0a | 0.7±0b |
| $T_3$ | 11.3±0.1b | 6.5±0.1c | 22±1c | 1.0±0.0c | 1.0±0.0c | 0.4±0c | 0.3±0c |
| $T_4$ | 9.5±0.0c | 5.4±0.1d | 17±1d | 0.6±0.0d | 0.7±0.0d | 0.3±0d | 0.1±0d |
| 120 days-growth | | | | | | | |
| $T_1$ | 29.3±0.2b | 21.5±0.2b | 65±2b | 3.9±0.1b | 2.4±0.0b | 1.8±0b | 1.0+0b |
| $T_2$ | 32.3±0.1a | 22.8±0.1a | 77±2a | 4.6±0.9a | 4.1±0.0a | 2.0±0a | 1.7+0a |
| $T_3$ | 29.3±0.4b | 19.8±0.1c | 65±2b | 3.0±0.0c | 2.4±0.1b | 1.1±0c | 1.0+0b |
| $T_4$ | 24.1±0.2c | 16.3±0.1d | 55±1c | 1.9±0.0d | 1.7±0.0c | 0.8±0d | 0.8+0c |

**Note:** SH-Shoot Height, RH-Root Height, NL-Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. $T_1$-*A. abyssinica* of AMF, $T_2$-*A. seyal* AMF, $T_3$-*A. tortilis* and $T_4$-Control (Non-Inoculum). At each column, and for each sampling date, same letter of rows indicates not significant differences in values among inoculation treatments (Tukey's test, P<0.05). Mean ± Standard error

**Table 3. Effect of arbuscular mycorrhizal inoculation on growth characteristics of *Delonix regia.***

| | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| 60 days-growth | SH (cm) | RH (cm) | NL | FSM (g) | FRM (g) | DSM (g) | DRM (g) |
| Treatment | | | | | | | |
| $T_1$ | 14.3±0.1a | 13.0±0.2b | 181±6b | 2.0±0.0a | 1.3±0.0b | 1.0±0.0a | 0.7±0a |
| $T_2$ | 15.0±0.2a | 13.8±0.1a | 204±2a | 2.1±0.0a | 1.6±0.0a | 1.0±0.0ab | 0.7±0a |
| $T_3$ | 14.0±0.2a | 13.2±0.1ab | 164±7b | 2.0±0.1a | 1.2±0.0b | 0.9±0.0b | 0.5±0a |
| $T_4$ | 11.6±0.1b | 12.0±0.0c | 120±1c | 1.4±0.0b | 0.9±0.0c | 0.7±0.0c | 0.3±0b |
| 120 days-growth | | | | | | | |
| $T_1$ | 19.3±0.1b | 18.0±0.2b | 242±1b | 3.5±0.1b | 3.0±0.0b | 2.1±0.0b | 1.9±0b |
| $T_2$ | 23.5±0.2a | 20.5±0.2a | 296±3a | 4.6±0.1a | 3.6±0.1a | 2.7±0.1a | 2.2±0a |
| $T_3$ | 17.8±0.1c | 16.1±0.1c | 231±1c | 3.9±0.2b | 3.0±0.0b | 2.2±0.0b | 1.8±0b |
| $T_4$ | 15.2±0.1d | 12.5±0.2d | 187±1d | 2.5±0.0c | 2.1±0.0c | 1.6±0.0c | 1.1±0c |

**Note:** SH-Shoot Height, RH-Root Height, NL-Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. $T_1$-*A. abyssinica* of AMF, $T_2$-*A. seyal* AMF, $T_3$-*A. tortilis* and $T_4$-Control (Non-inoculated). At each column, and for each sampling date, same letter of rows indicates not significant differences in values among inoculation treatments (Tukey's test, P<0.05). Mean ± Standard error

**Table 4. Effect of microbial inoculums on growth characteristics of *Sesbania grandiflora*.**

| 60 days-growth | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | SH (cm) | RH (cm) | NL | FSM (g) | FRM (g) | DSM (g) | DRM (g) |
| Treatment | | | | | | | |
| $T_1$ | 37.3±0.4a | 14.2±0.1b | 243±3.0a | 4.1±0.0b | 1.1±0.0b | 2.9±0.5ab | 0.6±0b |
| $T_2$ | 38.5±0.2a | 15.8±0.3a | 250±10.0a | 4.4±0.0a | 1.4±0.0a | 4.1±0.1a | 0.8±0a |
| $T_3$ | 37.6±0.4a | 15.3±0.1a | 226±4.0a | 4.0±0.0b | 1.3±0.1ab | 2.4±0bc | 0.6±0b |
| $T_4$ | 30.6±0.6b | 12.6±0.0c | 163±9.0b | 3.6±0.0c | 0.7±0.0c | 1.3±0.1c | 0.3±0c |
| 120 days-growth | | | | | | | |
| $T_1$ | 99.5±0.5b | 20.8±0.4b | 946±3.0b | 27.3±0.3b | 6.7±0.1b | 6.3±0.1b | 2.1±0b |
| $T_2$ | 118.0±2.0a | 26.3±0.6a | 1024±9.2a | 32.2±0.6a | 8.0±0.0a | 7.4±0.2a | 2.7±0a |
| $T_3$ | 100.4±0.3b | 23.0±0.5b | 950±1.1b | 28.0±0.5b | 6.9±0.0b | 6.8±0.0ab | 2.2±0b |
| $T_4$ | 85.0±3.5c | 17.9±0.2c | 733±8.8c | 23.0±0.5c | 5.2±0.0c | 4.5±0.1c | 1.6±0c |

**Note:** SH-Shoot Height, RH-Root Height, NL-Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. $T_1$-*A. abyssinica* of AMF, $T_2$-*A. seyal* AMF, $T_3$-*A. tortilis* and $T_4$ -Control (Non-Inocululated). At each column, and for each sampling date, same letter of rows indicates not significant differences in values among inoculation treatments (Tukey's test, $P<0.05$). Mean ± Standard error

*tortilis* ($T_3$) also showed significantly greater growth response in all parameters than the control ($T_4$) seedlings ($p<0.05$).

***Cassia fistula* L.**

At 60 and 120 days-growth, all plants inoculated with AMF showed significantly greater responses at all growth parameters over the non-inoculated control plants ($T_4$) (Table 5). Plants of *C. fistula* inoculated with AMF of *A. seyal* ($T_2$) showed significantly greater responses in all parameters over plants inoculated with AMF of $T_1$ and $T_3$. In general, similar growth increases were obtained for plants inoculated with $T_1$ and $T_3$.

**AMF root colonization and spore density**

Arbuscular mycorrhizal fungal structures were found associated with all root systems of inoculated plants. However, although non-sterile soil was used, no root colonization nor AMF spores were detected on non-inoculated plants or substrate, respectively. The percentage of AMF root colonization showed variability among the treatments and the trees host-plants (Table 6). In general, highest TC was found in roots of plants inoculated with AMF of *A. seyal* ($T_2$). Furthermore, *S. grandiflora* was the host plants which roots formed highest mycorrhizae.

Spore density of AMF recorded in the growth substrate showed differences among host plants. The mean SD value recorded in plants of *S. grandiflora* was significantly greater than the other host plants. Furthermore, plants of *S. grandiflora* inoculated with AMF of *A. abyssinica* ($T_1$) resulted in highest SD in their substrate, plants of *Delonix regia* inoculated with AMF of *A. seyal* ($T_2$) resulted in highest SD in their substrate, and plants of *A. indica* did not show differences of SD among inoculation treatments (Table 6).

**Table 5. Effect of arbuscular mycorrhizal inoculation on growth characteristics of *Cassia fistula*.**

| | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| 60 days-growth | SH (cm) | RH (cm) | NL | FSM (g) | FRM (g) | DSM (g) | DRM (g) |
| Treatment | | | | | | | |
| $T_1$ | 18.7±0.1b | 12.3±0.3b | 83±2b | 2.4±0.0b | 1.5±0.0a | 1.1±0.0b | 0.6±0ab |
| $T_2$ | 20.3±0.2a | 13.3±0.1a | 89±2a | 2.8±0.0a | 1.7±0.1a | 1.3±0.0a | 0.9±0a |
| $T_3$ | 17.8±0.1c | 12.0±0.2c | 78±1c | 2.5±0.0ab | 1.4±0.0a | 1.1±0.0ab | 0.5±0b |
| $T_4$ | 12.8±0.1d | 10.5±0.2d | 50±1d | 1.3±0.0c | 1.0±0.0b | 0.6±0.0c | 0.2±0c |
| 120 days-growth | | | | | | | |
| $T_1$ | 34.3±1.2b | 14.3±0.3b | 120±6a | 10.2± 0.1c | 2.9±0.0c | 4.7±0.1b | 2.1±0b |
| $T_2$ | 42.3±1.4a | 16.9±0.0a | 132±1a | 13.2± 0.1a | 4.5±0.0a | 5.4±0.0a | 2.3±0a |
| $T_3$ | 39.0±0.5ab | 16.8±0.5a | 124±2a | 11.0± 0.0b | 4.1±0.0b | 4.8±0.1b | 2.0±0c |
| $T_4$ | 25.6±0.8c | 11.3±0.1c | 69±1b | 8.9± 0.0d | 2.1±0.0d | 2.0±0.0c | 0.9±0d |

**Note:** SH-Shoot Height, RH-Root Height, NL-Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. $T_1$-*A. abyssinica* of AMF, $T_2$- *A.seyal* AMF, $T_3$-*A.tortilis* and $T_4$-Control (Non-Inoculated). At each column, and for each sampling date, same letter of rows indicates not significant differences in values among inoculation treatments (Tukey's test, $P<0.05$). Mean ± Standard error

**Table 6. Effect of AMF inoculation on total colonization of *A. indica, D. regia, S. grandiflora,* and *C. fistula* at 60 and 120 days-growth and spore density of plant growth substrate.**

| Host plant species | Inoculation Treatment | TC (%) 60 days-growth | TC (%) 120 days-growth | SD (soil $g^{-1}$) |
|---|---|---|---|---|
| *Sesbania grandiflora* | $T_1$ | 42.1±3.4b | 71.0±1.1a | 56.1±0.3a |
| | $T_2$ | 56.1±0.6a | 71.0±1.1a | 45.4±0.1b |
| | $T_3$ | 20.2±3.6c | 45.2±3.9b | 35.2±0.2c |
| Mean value | | 39.5±10B | 62.4±1.0A | 45.6±10A |
| *Azadirachta indica* | $T_1$ | 30.8±0.0a | 37.4±1.1b | 35.3±0.3a |
| | $T_2$ | 33.9±1.2a | 43.9±0.7a | 39.8±0.2a |
| | $T_3$ | 18.5±1.8b | 30.8±1.0c | 36.3±1.7a |
| Mean value | | 27.7 ±8.0C | 37.4±6.0A | 37.1±1.0B |
| *Delonix regia* | $T_1$ | 25.7±0.0b | 57.6±0.6b | 35.3±0.2b |
| | $T_2$ | 35.1±1.9a | 62.8±0.0a | 40.2±0.1a |
| | $T_3$ | 26.9±0.7b | 33.7±0.2c | 32.6±0.2c |
| Mean value | | 29.3±2.0C | 54.4±8.0C | 36.0±2.0B |
| *Cassia fistula* | $T_1$ | 45.2±1.0a | 53.4±0.4c | 29.7±0.2b |
| | $T_2$ | 49.8±2.2a | 61.9±0.2a | 33.2±0.3a |
| | $T_3$ | 45.9±3.1a | 58.8±0.7b | 29.4±0.3b |
| Mean value | | 47.0±1.0A | 58.0±2.0B | 30.8±1.0C |

**Note:** TC-Total Colonization, SD-Spore Density. At each column, and for each sampling date, different lowercase letters denote, for the same host plant, differences between inoculation treatments. Different uppercase letters denote differences, in average values, between host plants. (Tukey's test, $P<0.05$).

**Mycorrhizal dependency**

At 60 days of growth, *C. fistula* plants showed the highest mycorrhizal dependence (MD), however, this response to mycorrhization changed and, at 120 days of growth, the highest MD was recorded in *S. grandiflora* plants (Table 7). On the other hand, it should be noted that in the two growth stages evaluated, the highest MD was recorded in the plants inoculated with AMF of *A. seyal* ($T_2$).

**Table 7. Mycorrhizal dependency of tree plants inoculated with arbuscular mycorrhizal fungi indigenous from Ethiopia.**

| Inoculation Treatment | MD % 60 d-growth | MD % 120 d-growth |
|---|---|---|
| $T_1$ | 35.99b | 54.90b |
| $T_2$ | 43.75a | 59.99a |
| $T_3$ | 27.92c | 42.17c |
| Host plant species | | |
| *Sesbania grandiflora* | 39.50b | 62.40a |
| *Azadirachta indica* | 27.70c | 37.40d |
| *Delonix regia* | 29.30c | 54.40c |
| *Cassia fistula* | 47.00a | 58.00b |

**Note:** MD- mycorrhizal dependency, $T_1$-AMF from *A. abyssinica*, $T_2$-*A. seyal*, $T_3$-*A. tortilis*. At each column, and for each sampling date, same lowercase letter of rows indicates not significant differences in values among inoculation treatments (Tukey's test, $P<0.05$).

**Correlation between the parameters**

Pearson's correlation revealed that all plant growth parameters in each seedling showed significant strong and positive correlations (Table 8). Likewise, significantly positive associations between AMF root colonization and SD and the plant growth parameters were also found, except in *A. indica* in which the association between SD and FRM; SD and DRM were positive but not significant ($P>0.05$) (Table 8).

## DISCUSSION

The current study was focused on evaluating the effects of AMF inoculation isolated from soil associated to roots of acacia trees growing at highland and lowland areas of Ethiopia, to improve the growth and survival of the seedlings of the selected forest trees (*A. indica, D. regia, S. grandiflora* and *C. fistula*) under low fertility and acidic soil. After multiplication in trap cultures, the mycorrhizal propagules associated with roots of *A. seyal*, native from Batu and Bishoftu, produced the greatest multiplication, which was revealed both by the colonisation of the roots of the trap cultures and by the abundance of spores in the multiplication substrate. Similarly, plants inoculated with mycorrhizal propagules associated with roots of *A. seyal* showed, in general, the best performance in all growth parameters evaluated. This result was also observed in the study reported by Belay *et al*. (2013) who concluded that *A. seyal* is characterized by relatively high AMF colonization and AMF

diversity compared to the other acacia species. Differences in AMF mycorrhization between the acacia species could be due to factors such as climatic and edaphic properties, host-specificity between fungi and plants, age of the host plants, disturbance, and differential sporulation ability of AMF taxa (Song *et al*., 2019; Vieira *et al*., 2019; Ma *et al*., 2023), that are specific to each site where soil associated to roots samples for AMF multiplication were collected. Yang *et al*. (2012) also reported that the distribution of AMF showed a pattern of high endemism at large scales. This pattern indicates high specificity of AMF for hosts at different scales (plant taxonomic order and functional group) and high selectivity from host plants for AMF.

All inoculated plants showed higher performance in their growth parameters than non-inoculated plants. This performance was associated with the formation of mycorrhizae as a result of inoculation. The absence of mycorrhizal and spore-forming propagules in the non-inoculated control plants highlights the need to reinforce microbial populations, particularly mycorrhizal fungi, in studied

**Table 8. Pearson correlation coefficients between the growth parameters, total colonization and spore density of trees inoculated with arbuscular mycorrhizal fungi.**

| | SH (cm) | RH (cm) | NB | FSM (g) | FRM (g) | DSM (g) | DRM (g) | TC | SD |
|---|---|---|---|---|---|---|---|---|---|
| *Azadirachta indica (neem)* | | | | | | | | | |
| SH | 1 | | | | | | | | |
| RH | 0.935** | 1 | | | | | | | |
| NL | 0.916** | 0.874** | 1 | | | | | | |
| FSM | 0.925** | 0.964** | 0.911** | 1 | | | | | |
| FRM | 0.881** | 0.807** | 0.922** | 0.862** | 1 | | | | |
| DSM | 0.831** | 0.936** | 0.814** | 0.966** | 0.794** | 1 | | | |
| DRM | 0.833** | 0.780** | 0.910** | 0.842** | 0.987** | 0.790** | 1 | | |
| HC | 0.877** | 0.870** | 0.707* | 0.778** | 0.582* | 0.686* | 0.498 | 1 | |
| SD | 0.752** | 0.896** | 0.629* | 0.854** | 0.509 | 0.865** | 0.469 | 0.840** | 1 |
| *Delonix regia* | | | | | | | | | |
| SH | 1 | . | | | | | | | |
| RH | 0.961** | 1 | | | | | | | |
| NL | 0.991** | 0.973** | 1 | | | | | | |
| FSM | 0.876** | 0.887** | 0.905** | 1 | | | | | |
| FRM | 0.944** | 0.967** | 0.963** | 0.956** | 1 | | | | |
| DSM | 0.930** | 0.897** | 0.941** | 0.951** | 0.958** | 1 | | | |
| DRM | 0.894** | 0.936** | 0.925** | 0.871** | 0.942** | 0.852** | 1 | | |
| TC | 0.707* | 0.823** | 0.772** | 0.809** | 0.852** | 0.730** | 0.911** | 1 | . |
| SD | 0.795** | 0.894** | 0.850** | 0.889** | 0.923** | 0.830** | 0.941** | 0.963** | 1 |
| *Sesbania grandiflora* | | | | | | | | | |
| SH | 1 | | | | | | | | |
| RH | 0.928** | 1 | | | | | | | |
| NL | 0.897** | 0.881** | 1 | . | | | | | |
| FSM | 0.934** | 0.947** | 0.912** | 1 | | | | | |
| FRM | 0.951** | 0.940** | 0.961** | 0.952** | 1 | | | | |
| DSM | 0.917** | 0.923** | 0.955** | 0.942** | 0.960** | 1 | . | | |
| DRM | 0.953** | 0.968** | 0.916** | 0.962** | 0.971** | 0.933** | 1 | | |
| TC | 0.743** | 0.742** | 0.941** | 0.806** | 0.852** | 0.882** | 0.765** | 1 | |
| SD | 0.889** | 0.852** | 0.988** | 0.912** | 0.952** | 0.944** | 0.890** | 0.954** | 1 |
| *Cassia fistula* | | | | | | | | | |
| SH | 1 | | | | | | | | |
| RH | 0.926** | 1 | | | | | | | |
| NL | 0.930** | 0.925** | 1 | | | | | | |
| FSM | 0.908** | 0.849** | 0.814** | 1 | | | | | |
| FRM | 0.943** | 0.939** | 0.849** | 0.925** | 1 | | | | |
| DSM | 0.915** | 0.879** | 0.960** | 0.821** | 0.867** | 1 | | | |
| DRM | 0.899** | 0.865** | 0.973** | 0.818** | 0.817** | 0.983** | 1 | . | |
| TC | 0.878** | 0.863** | 0.977** | 0.735** | 0.781** | 0.972** | 0.983** | 1 | |
| SD | 0.920** | 0.885** | 0.978** | 0.834** | 0.854** | 0.994** | 0.993** | 0.979** | 1 |

Note: SH-Shoot Height, RH-Root height, NL-Number of leaves, FSM-Fresh Shoot Mass, FRM-Fresh Root Mass, DSM-Dry Shoot Mass, DRM-Dry Root Mass, TC-Total Colonization, and SD–Spore Density. ** Correlation is significant at the 0.01 level (2-tailed) and * Correlation is significant at the 0.05 level (2-tailed)

areas. From the outcome of the experiment, it can be inferred that AMF inoculation in unsterile soil, although with low-null active AMF populations, definitely boosted the growth of the seedlings. The results obtained, although not surprising, are very promising considering that they could be used for reforestation plans in Ethiopia. Decades ago, a greenhouse experiment was conducted in *S. grandiflora* by Habte and Aziz (1985), who found that nutrient uptake and growth of *S. grandiflora* in nonsterile soil was significantly stimulated by inoculation of soil with AMF. Another study by Banerjee *et al*. (2013) in India showed clear evidence of positive growth responses after AMF inoculation with AMF associated with roots of *A. indica* (Neem). They found that the screening of efficient AMF for *A. indica* under nursery conditions showed that significant increases in the growth parameters and phosphorous uptake was found for most of the AMF species against control. However, a recent experiment that carried out in Ogun State, Nigeria indicated that the interaction of mycorrhiza and moisture supply had no significant effect on the seedling growth of *C. fistula* (Oladipo *et al*., 2021). Similarly, Gehring and Connell (2006) examined the occurrence and levels of AMF colonization of some common seedling species in a tropical and a subtropical rain forest site in Queensland, Australia. They found that seedling survival was significantly positively associated with seed biomass but not with AMF colonization.

*Sesbania grandiflora* was the host plant species that showed the highest mycotrophic capacity (formation of highest mycorrhizal colonization) as well as the highest mycorrhizal dependence at the end of the period of study. It should be noted that in early stages (60 days of growth) *C. fistula* also showed high mycorrhizal formation ability and also high mycorrhizal dependence. So that, these two species could be considered good candidates for reforestation programmes. Furthermore, the legume seedlings (*S. grandiflora, C. fistula* and *D. regia*) treated with AMF showed higher percentage of root colonization by AMF, MD and spore density than *A. indica* the non-leguminous tree (Table 6 and 7). This could be due to the fact that legumes require more P than non-leguminous plants because the maintenance of biological $N_2$ fixation process in root nodules is highly dependent on P (Plenchette, 1983).

In addition, and as mentioned, the microflora together with the AMF associated with *A. seyal* was the inoculum with which the best performance was achieved in all the growth parameters evaluated in the trees. Future studies should, at first, confirm the results obtained in overwintering under field conditions. Furthermore, the identification (by classical and/or molecular taxonomy) of both the AMF and associated microbiota that belong the consortium associated with *A. seyal* should be carried out. The present research also revealed that all plant growth parameters were positively correlated with mycorrhizal formation. Accordingly, significantly and positive associations between mycorrhizal parameters and the plant growth parameters such as total dry biomass, number of leaves, root height, shoot height, fresh shoot mass, fresh root mass, dry shoot mass, and dry root mass were obtained (Table 8). This confirms the effect of mycorrhizae formation enhanced by inoculation on the growth of the studied trees. Additionally, very strong positive correlations ($p \leq 0.01$) in AMF spore density with all plants' growth parameters were found. Likewise, except for *D. regia*, the AMF root colonization in all plants was also found to have very high positive relationships ($p \leq 0.01$) with the growth parameters in all plant varieties. This could suggest that the growth benefit at *D. regia* was due to the large extracellular hyphae AMF network, which transports water and nutrients to roots, increasing their absorption range and helping plants in the low nutrient soil (Pei *et al*., 2020). To confirm this, future studies should quantify the extent of fungal mycelium on inoculated plants, as well as its effect on improving soil structure. AMF root colonization was also found to have very high positive relationships ($p \leq 0.01$) with AMF spore density in the substrate. Other studies also reported positive correlations between spore numbers and root colonization (Songachan *et al*., 2011; Sivakumar, 2013; Birhane *et al*., 2020). However, Salim *et al*. ( 2020) found that AMF root colonization had a negative relationship with the number of spores in the soil during their evaluation of the status of colonization of the roots of the host plant in various age classes of revegetation of post-coal mining land associated with AMF spores populations and soil fertility in Indonesia. In our study, this would indicate that AMF colonization had a favorable impact on the growth parameters of selected tree seedlings and their mycorrhizal dependence, as well as on the spore density in the growth substrate.

Sometimes, AMF propagules in fresh soil (mainly in degraded soils) consist mainly of segments of AMF hyphae and colonized roots, rather than viable spores (Lugo and Cabello, 2002; Troeh and Loynachan, 2009; Thougnon Islas *et al*., 2016; Covacevich *et al*., 2021) that can be used in inoculation schemes. In our study, multiplication in field soil on trap plants yielded AMF spores (and other propagules) that were able to germinate, colonize and promote growth of the studied tree host plants. Future studies should consider the establishment of large-scale multiplication of the most promising AMFs to achieve inoculum quantities needed for field inoculation programs.

## CONCLUSIONS

From the above results, it can be concluded that inoculum with AMF from soil associated to roots of *A. seyal* native from lowlands, followed by *A. abyssinica* from highland, were found to be the best sources of inoculum to improve greatly the seedlings establishment and performance of tree grown in poor fertile and low pH soil. Therefore, AMFs associated with these host plant species can be considered potential candidates for inoculation programs than can be adopted as regular practices at different sites of nurseries in Ethiopia in improving the survival and growth of seedlings in the national tree seedling planting campaigns. However, future studies should aim at evaluating (i) the potential of these inoculants to environmental stress under the field conditions and, (ii) the competitiveness of these native inoculums with commercial microbial inoculants (iii) the genetic identity of AMFs and the accompanying microbiota in inoculants.

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**Compliance with ethical standards**. The nature of this work does not require approval by a (bio) ethical committee.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon the reasonable request.

**Author contribution statement (CRediT). Y. Legesse-**investigation, writing original draft, **M.L. Puente-**writing-review and editing, **F. Covacevich-**writing-review and editing, **M. Jida-**project management, writing-review and editing, **Z. Belay-**conceptualization, methodology, writing-review and editing.

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