BIODIVERSITAS
Volume 22, Number 5, May 2021
Pages: 2971-2980

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220562

Cordia africana but not *Juniperus procera* and *Podocarpus falcatus* respond positively to arbuscular mycorrhizal fungi at the early stages of seedling development

FISSEHA ASMELASH[1,2,♥], TAMRAT BEKELE[2], ZERIHUN BELAY[3], FASSIL KEBEDE[4]

[1]Forest and Rangeland Plants Biodiversity Directorate, Ethiopian Biodiversity Institute. P.O.Box: 30726, Addis Ababa, Ethiopia, [♥]email: fisseha33@gmail.com
[2]Department of Plant Biology and Biodiversity Management, Addis Ababa University. Addis Ababa, Ethiopia
[3]Department of Applied Biology, School of Natural Sciences, Adama Science and Technology University. Adama, Ethiopia
[4]Center for Soil and Fertilizer Research in Africa, Mohammed VI Polytechnic University. Benguerir, Morocco

Manuscript received: 24 March 2021. Revision accepted: xxx April 2021.

Abstract. *Asmelash F, Bekele T, Belay Z, Kebede F. 2021.* Cordia africana *but not* Juniperus procera *and* Podocarpus falcatus *respond positively to arbuscular mycorrhizal fungi at the early stages of seedling development. Biodiversitas 22: 2971-2980.* AMF (Arbuscular mycorrhizal fungi) inoculation could be an important technology to improve the growth and field survival of trees. Hence, we evaluated the mycorrhizal responsiveness of *Cordia africana* Lam., *Juniperus procera* (Hoechst. ex Endl.), and *Podocarpus falcatus* (Thumb.) Mirb. seedlings. Seedlings germinated on sterile sand were transplanted to 1-liter plastic pots filled with sterile and non-sterile degraded bulk soil. Rhizospheric soil from adult *C. africana* and *J. procera* were used as whole-soil AMF inocula. *Cordia africana* and *J. procera* received conspecific whole-soil AMF inocula while *P. falcatus* received *J. procera* inoculum. Hence, in the two-by-two factorial experiment, we also evaluated the growth effects of AMF inoculation, soil type, and their interaction. On the sterile potting soil, MRi (mycorrhizal responsiveness due to AMF inoculation) of *C. africana* was positive and significantly (p<0.05) greater than the MRi of *J. procera* and *P. falcatus*. However, on the non-sterile potting soil, it was significantly greater than the MRi of *P. falcatus* only. MRs (MR due to the existing potting soil inocula) and considering all growth variables were mostly positive for *C. africana* but negative for *J. procera* and *P. falcatus*. AMF inoculation significantly increased most growth variables of *C. africana* seedlings and no significant "inoculation" x "soil type" interaction effects were detected. Hence, AMF inoculation of *C. africana* seedlings could be merited and under wide range of field conditions. In the case of *J. procera* and *P. falcatus*, after-planting care could be more appropriate.

Keywords: arbuscular mycorrhizal fungi, AMF, *Cordia africana*, forest restoration, mycorrhizal responsiveness, relative growth rate

INTRODUCTION

Restoration of the dry evergreen Afromontane forests (DAF) has long been among the primary environmental agendas in Ethiopia (Bishaw 2001; Asmelash et al. 2021). Currently, DAF restoration is also considered to be a global forest restoration priority (Strassburg et al. 2020). Afromontane soils are too deficient in the essential nutrients (e.g. N&P) required for seedlings' survival, growth, and forest development (Dalling et al. 2016). DAF restoration also requires the planting of DAF characteristic tree species (Lemenih and Teketay 2004; Aerts et al. 2007) which, for successful field establishment, require after-planting care (mulching, watering, hoeing, weeding, and manure application) for up to 3 years (Negash 2010). Hence, due mainly to these reasons, DAF restoration is very challenging.

Arbuscular Mycorrhiza (AM) is an adaptive symbiosis that plants initiate under nutrient and moisture stress (Smith and Read 1998; Gutjahr 2014). The arbuscular mycorrhizal fungi (AMF) develop extraradical mycelia which are very extensive, able to permeate into soil microsites (Leake et al. 2004). Hence, under poor soil nutrient and moisture conditions, AMF could significantly increase planted seedlings' access to essential nutrients and moisture, and hence, increase their growth, field survival, and establishment (Schüßler et al. 2016). Therefore, AMF inoculation could be relevant biotechnology in DAF restoration programs (Asmelash et al. 2016).

Previously, Asmelash et al. (2019) carried out a field inoculation experiment on *Cordia africana* Lam., *Juniperus procera* (Hoechst. ex Endl.), and *Podocarpus falcatus* (Thumb.) Mirb. and reported no significant inoculation effect on the survival of neither of the species. However, the experiment was carried out on fertile soil and only a few growth variables were measured. Moreover, the seedlings were obtained from nearby nurseries and could already have sufficient inoculum. According to Verbruggen et al. (2013), AMF inoculation success or failure greatly depends on, among others, the AMF status of the planting sites and planted seedlings. This study was therefore carried out by improving the experimental design of the previous experiment. Accordingly, in this study, severely degraded soil was used as a substrate and up to twelve growth variables were considered as compared to only two in the previous experiment (Asmelash et al. 2019). Moreover, the AMF status of the substrate and seedlings were determined. Hence, seedlings were germinated and root trained on sterile sand, AMF spore abundance of the substrate was determined and seedlings' AMF root

colonization levels were determined. The study was conducted to know (i) if the simple technique of whole-soil AMF inoculation could improve the growth of *C. africana*, *J.procera*, and *P. falcatus* seedlings on degraded DAF ecosystem soil, (ii) if whole-soil AMF inoculation could be important on non-sterile potting soils which already have AMF inoculum and resemble field conditions, (iii) how the early, mid, and late-successional trees, viz., *C. africana*, *J.procera*, and *P. falcatus* respond to AMF.

MATERIALS AND METHODS

The experimental plant species

Cordia africana Lam. is a broad-leaved deciduous tree species while *Juniperus procera* (Hoechst. ex Endl.) and *Podocarpus falcatus* (thumb.) Mirb. are dioecious conifers (Negash 2010). *Podocarpus falcatus* is heterorhizic and has two distinct root forms, viz., the long indeterminate roots and determinate fine nodules or the modified root hairs (Figure 1; Baylis et al. 1963).

We selected *C. africana, J. procera* and *P. falcatus* for this study since, (i) they are known to be arbuscular mycorrhizal (Asmelash et al. 2019), (ii) they are important for DAF restoration, i.e., *C. africana* is among the most widely raised native tree species in those nurseries suitable for DAF restoration (Asmelash et al. 2021) and *J. procera* and *P. falcatus* are characteristic DAF tree species (Friis et al. 2010), and (iii) they represent different successional groups, viz., *C. africana* is early successional (Friis 1992; Yirdaw et al. 2002) while *J. procera* and *P. falcatus* respectively are mid and late-successional tree species (Teketay 1997; Abebe et al. 2010).

Seedling preparation and inoculation

Seeds were prepared, sown, and root trained following the procedures outlined by Negash (2010). Before sowing the *P. falcatus* seeds, the sclerotesta were carefully broken by using basalt stone. Seeds with the sclerotesta removed were immediately soaked in 5% household bleach for 10 minutes and further soaked in household food-grade vinegar for another 5 minutes (to neutralize the base). Finally, they were thoroughly washed using tap water and then soaked in distilled water for 20 hours to initiate imbibition. The water was decanted and seeds, with their mouth in the upside direction, were sown on plastic pots (7 cm diameter and 8 cm height) filled with sterile sand. Seeds were sown by inserting the mouth just below the sand surface. The seeds of *C. africana* and *J. procera* were cleaned similarly to that of *P. falcatus* and were also sown by drilling them in the sterile sand. For *P. falcatus* and *J. procera* respectively, 2 and 3 seeds were sown per pot. The sand was sterilized by autoclaving for 30 minutes in two cycles of 24 hours intervals (Negash 2010).

When seedlings developed well enough, 24 individuals with comparable sizes were selected per species for inoculation. AMF inoculation was done when transplanting these seedlings to the 1-liter plastic pots. The potting soil used in this experiment was a degraded bulk soil collected from within the degraded gaps of the Ankorcha dry evergreen Afromontane forest, Addis Ababa. Currently, Ankorcha forest is mainly composed of *Eucalyptus* trees but it also has the native tree and shrub species including *J. procera*, *Olea europaea*, and *Dovyalis abyssinica.* The potting soil is severely degraded with pH = 6.245±0.015, EC = 32.35±0.35dS/m, TN = 0.07%, P (Bray-II) = 4.74 ppm, OM = 5.36%, CEC = 19.04mequi/100 g. The AMF spore abundance was quantified to be 13.95±1.6 g^{-1}. The main reason for collecting the potting soil from Ankorcha was due to the fact, it represents degraded DAF ecosystem soils.

Rhizospheric soils at a depth between 10 and 30 cm were collected from *C. africana* and *J. procera* adult trees at a distance of 1 m further away from the trunks (Zarik et al. 2016) and were used as whole-soil AMF inocula. Since there were no *C. africana* and *P. falcatus* individuals found in Ankorcha forest and environ, *C. africana* seedlings were inoculated with *C. africana* rhizospheric soil collected in the Ethiopian Biodiversity Institute (EBI). Moreover, *P. falcatus* and *J. procera* seedlings were inoculated with rhizospheric soil of *J. procera* collected from Ankorch forest. Therefore, whereas *C. africana* and *J. procera* received conspecific inoculum, *P. falcatus* was inoculated with a non-conspecific inoculum.

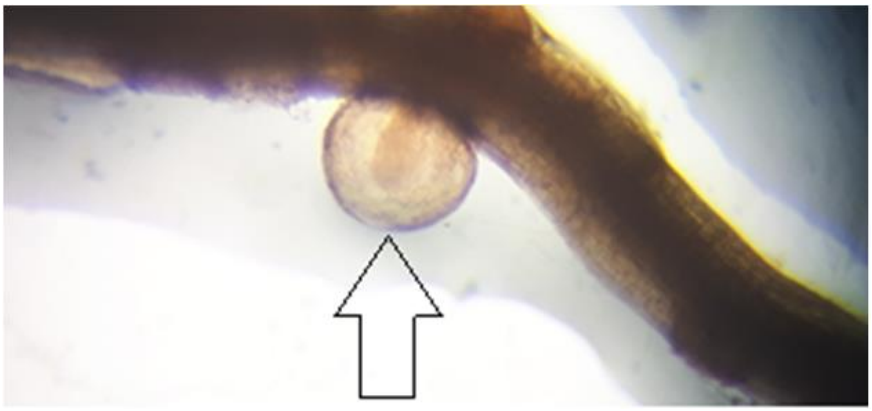

Figure 1. A microscopic view of *Podocarpus falcatus* (thumb.) Mirb. fine root with the nodule indicated by the arrow

Figure 2. Transplanted seedlings of *Juniperus procera* (Hoechst. ex Endl.), *Cordia africana* Lam., and *Podocarpus falcatus* (thumb.) Mirb. (from *left* to *right*)

Experimental setup

The experiment was carried out in the EBI mesh-house. Mesh-house experiment rather than greenhouse or field experiment was preferred because it has the advantage of mimicking the filed condition better than greenhouse while it in the meantime enables the much-needed control of the experiment better than field experiments which can be chaotic.

The experiment was a two-by-two factorial with two levels of AMF inoculation (+AMF and –AMF), two types of substrate (sterile and non-sterile potting soil) in six replications. The total number of seedlings used was therefore 72 (24 per species) (Figure 2). Seedlings with "+AMF" received 45 g whole-soil AMF inoculum while those with "–AMF" received 40g heat sterilized whole-soil AMF inoculum and 40ml microbial filtrate (Onguene and Kuyper 2005). The microbial filtrate was obtained following the procedure outlined by Pánková et al. (2014). Both substrate soil and "–AMF" sterilization was done by heating in the oven for 30 minutes at 120°C in two cycles with 24 hours interval (Hart and Reader 2004).

Treatments were arranged in a completely randomized design (CRD). The experiment lasted from November 14, 2019- April 14, 2020 (for five months) for *C. africana*, November 14, 2019- April 28, 2020 (for five and half months) for *J. procera*, and September 26, 2019-March 25, 2020 (for six months) for *P. falcatus*. Watering was done every other day to field capacity, especially for the first three months. Afterward, seedlings were watered to field capacity every week. This was done, partly, to mimic the rainfall condition at the field in the DAF ecosystem.

AMF root colonization, mycorrhizal responsiveness, and seedlings growth variables determination

AMF root colonization (RC) was determined by the ink and vinegar technique (Vierheilig et al. 1998) followed by the gridline intersects method (Giovanetti and Mosse 1980) and using black hero ink (Asmelash et al. 2021). In order not to reduce the root weight, only five 1cm fine roots per seedlings or 30 cm fine root samples per treatment were used for RC determination. However, since the root system of *J. procera* was very small, RC was not determined.

AMF responsiveness (MR) was computed following Rowe et al. (2007) and Janos (2007). Hence, MR due to inoculation (MRi) was computed following Rowe et al. (2007) by the formula; MRi=*ln*([+AMF]/[-AMF]), where [+AMF] and [-AMF] represent the average dry mass of inoculated and non-inoculated seedlings respectively and "*ln*" is the natural logarithm. This formula was important in that it enabled statistical mean MRi comparison between tree species.

The non-sterile potting soil has native Ankorecha AMF inoculum and hence, could influence seedlings' growth. Therefore, using all the growth variables (including dry mass), MR due to the existing pot soil AMF inoculum (MRs) was also computed using the formula by Janos (2007), i.e., MRs= (nSnI)-(SnI)/(SnI)*100, where "nSnI" refers to the growth of seedlings on the non-sterile-non-inoculated potting soil and "SnI" refer to growth on the sterile-non-inoculated potting soil.

Seedling growth variables measured in this experiment were, fresh and dry shoot, root, and total masses, leaf number (LN), leaf area (LA), relative leaf number growth rate (RGR-LN), relative height growth rate (RGR-H), relative collar diameter growth rate (RGR-CD), rooting depth (RD), root to shoot (R:S) and root to plant (R:P) ratios. Hence, when initial measurements were available, growth variables were determined as relative growth rates.

Seedling height and rooting depth were measured by using a ruler. Collar diameter was determined by using a digital caliper. Seedlings' fresh and dry mass was measured by using an analytical balance. Root and shoot dry masses were determined respectively after oven drying at 70°C and 65°C to constant mass (Ouahmane et al. 2006). Leaf area was measured by the direct method of graph-paper tracing which is considered the most accurate way of determining leaf area (Pandey and Singh 2011), especially when the sample size is small and detaching leaves do not affect the experiment. Relative growth rate (RGR)was determined following Hunt (1990), by the formula $RGR= 1/X_i (\Delta X/\Delta T)$, where ΔX is the change in growth (X_f-X_i), X_f is the final measurement, X_i is the initial measurement, ΔT is the time for the change. We measured ΔT in weeks and was20, 22, and 24 weeks respectively for *C. africana*, *J. procera*, and *P. falcatus*.

Statistical analysis

Using the software SPSS version 20, one-way ANOVA and Tukey-HSD tests were computed to compare the MRi of *C. africana*, *J. procera*, and *P. falcatus* both on sterile and non-sterile potting soil. Two-way ANOVA also was computed to know the AMF inoculation, soil type, and AMF inoculation x soil type interaction effects on the AMF root colonization and growth of *C. africana*, *J. procera*, and *P. falcatus* seedlings.

RESULTS AND DISCUSSION

Mycorrhizal responsiveness (MR)

The mean mycorrhizal responsiveness due to whole-soil AMF inoculation (MRi) of *C. africana*, *J. procera*, and *P. falcatus*, was found to be 1.05±0.17, 0.4±0.09, and -0.02±0.09 respectively, on the sterile potting soil, while on the non-sterile potting soil, it was 0.83±0.12, 0.26±0.28, and 0.002±0.07 (Figure 3). The one-way ANOVA results indicated that there was a statistically significant difference between the MRi of the study species on both potting soil types (Table 1). The MRi of *C. africana* on the sterile potting soil was, significantly (p<0.05) and 167.64% greater than the MRi of *J. procera*, while the MRi of *P. falcatus* was also significantly and 102% lower than the MRi of *C. africana*. On the non-sterile potting soil, the MRi of *C. africana* was not significantly different from the MRi of *J. procera* but MRi of *P. falcatus* was significant, 99.76% lower than the MRi of *C. africana* (Figure 3).

Considering the existing Ankorcha forest soil AMF inoculum (potting soil inoculum), the mycorrhizal responsiveness (MRs) in the case of *C. africana*, was positive for all growth variables except root to shoot and root to plant dry mass ratios. However, in the case of *J. procera* and *P. falcatus*, the MRs was negative except leaf number and relative collar diameter growth rate of *P. falcatus* which were positive and zero respectively (Table 2).

Table 1. One-way ANOVA results for the mycorrhizal responsiveness of tree species due to AMF inoculation (MRi)

		Sum of squares	df	Mean square	F	*P*
MRiS	Between groups	3.510	2	1.755	18.922	0.0001***
	Within groups	1.391	15	0.093		
MRinS	Between groups	2.174	2	1.087	5.560	0.016*
	Within groups	2.933	15	0.196		

Note: MRiS: Mycorrhizal responsiveness on sterile potting soil, MRinS: Mycorrhizal responsiveness on non-sterile potting soil.* Significant at $p \leq 0.05$, *** significant at $p \leq 0.001$.

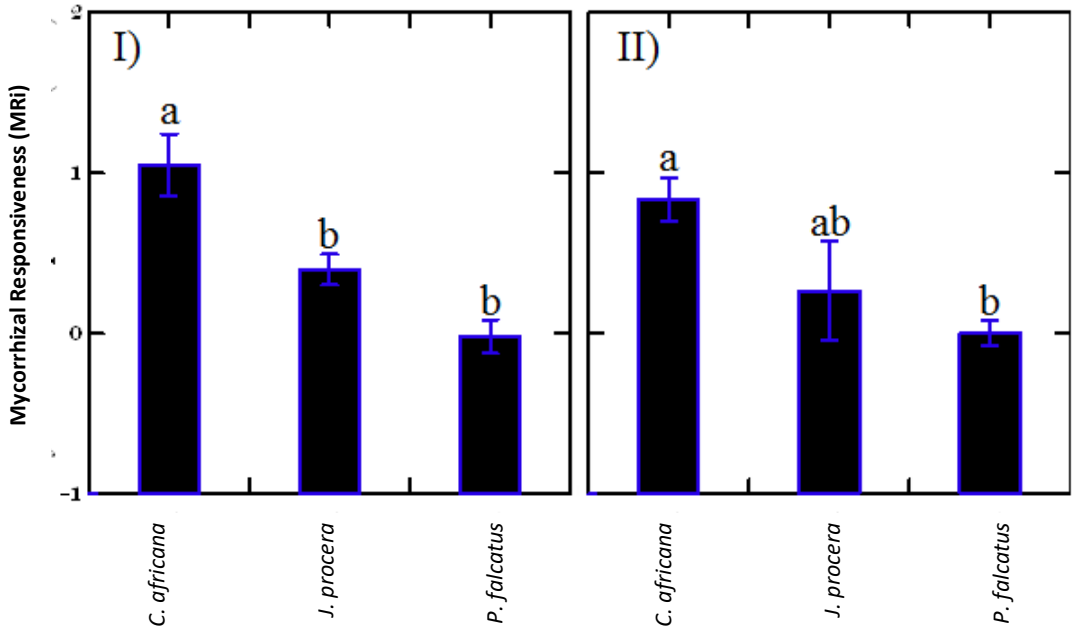

Figure 3. Pairwise comparison of the mycorrhizal responsiveness of *C. africana*, *J. procera*, and *P. falcatus* on sterile potting soil (I) and non-sterile potting soil (II). Means containing similar letters are not significantly different after Tukey HSD (p<0.05)

Figure 4. *Cordia africana* seedlings after 5 months of growth in the mesh-house. The difference in leaf number and area between treatments is visible. S&nI: sterile potting soil and seedlings not inoculated, S&I: sterile soil and seedlings inoculated, nS&nI: non-sterile soil and seedlings not inoculated, nS&I: non-sterile soil and seedlings inoculated with whole-soil AMF.

Table 2. Mycorrhizal responsiveness (%) of *C. africana*, *J. procera* and *P. falcatus* due to the existing Ankorcha forest soil AMF inoculum (MRs)

Growth variables	Tree species		
	C. africana	***J. procera***	***P. falcatus***
Leaf number	+163.9	-11.5	+1.2
Relative height growth rate (cm/week)	+23.53	-13.79	0
Relative collar diameter growth rate (mm/week)	+266.67	-27.27	-16.67
Shoot fresh mass (g)	+11.63	-15.14	-6.87
Shoot dry mass (g)	+54.55	-5.97	-13.15
Root fresh mass (g)	+21.62	-23.33	-32.43
Root dry mass (g)	+7.14	-29.92	-20.54
Plant fresh mass (g)	+16.67	-18.8	-18.6
Plant dry mass (g)	+15.4	-13	-16
Root to shoot dry mass ratio	-32.58	-35.2	-8.4
Root to plant dry mass ratio	-18.48	-28.9	-5.45

The effects of whole-soil AMF inoculation and soil type on seedlings' root colonization (RC)

AMF root colonization (RC) of *C. africana* was significantly influenced by the whole-soil AMF inoculation. It was also significantly influenced by the soil type and by AMF inoculation x soil type interaction. However, neither AMF inoculation, soil type, nor their interaction had significant effects on the RC of *P. falcatus*. As would be expected, non-inoculated seedlings on the sterile potting soil were not colonized by AMF. Whereas, the mean RC of whole-soil AMF inoculated *C. africana* seedlings were moderate to high (36.2-62.6%), it remained very low (4.17-6.67%) in the case of *P. falcatus* (Table 3).

The effects of whole-soil AMF inoculation and soil type on seedlings' growth

Whole-soil AMF inoculation, except for the significant effect it had on the relative collar diameter growth rate (RGR-CD) of *P. falcatus*, was found to have no significant effect on almost all the growth variables of both *J. procera* and *P. falcatus* seedlings. Significant soil type effects were found for leaf number and root fresh mass of *J. procera* and *P. falcatus*. Moreover, significant soil type x inoculation interaction effects were found for rooting depth in the case of *J. procera* and leaf number in the case of *P. falcatus*. On the contrary, whole-soil AMF inoculation was found to have significant effects on all of the growth variables of *C. africana* except relative height growth rate, rooting depth, Root: shoot, and Root: plant dry mass ratios (Table 3).

Whole-soil AMF inoculation resulted in a significant 376.17% and 311.11% increase in LAand RGR-LN of *C. africana* seedlings grown on the sterile potting soil and on the non-sterile potting soil also it has a significant and

379.03% and 320% increases respectively (Figure 4; Table 4.A). Inoculation also resulted in a significant 255.56% and 123.33% increases in RGR-CD of *C. africana* on the sterile and non-sterile potting soil respectively. Moreover, *C. africana* seedlings grown on the sterile potting soil had a significant and 202.11% more SfM after 5 months due to whole-soil AMF inoculation while those grown on the non-sterile potting soil had a significant and 143.15% more SfM. On the other hand, inoculation increased SdM of *C. africana* seedlings significantly by 207.96% when grown on the sterile pot soil and by a significant 116.76% when grown on the non-sterile potting soil. Inoculation also increased PfM and PdM of *C. africana* by a significant 96.63% and 198.8% respectively when growth on the sterile potting soil and by 121.86% % and 133.13% respectively when grown on the non-sterile potting soil (Table 4.A).

Juniperus procera seedlings grown on the non-sterile potting soil grew a significant 42% and 36.62% lower root and seedling fresh masses respectively compared to those seedlings grown on the sterile potting soil. Rooting depth was greater for the inoculated seedlings on the non-sterile soil while it was lower on the sterile soil (Table 4.B). On the other hand, *P. falcatus* seedlings also grew a significant 9.3% more number of leaves and 42.6% more root fresh mass on sterile potting soil compared to seedlings grown on non-sterile soil. Due to the inoculation x soil type interaction effect, while inoculated *P. falcatus* seedlings grew 16.24% more number of leaves on the sterile potting soil compared to the non-inoculated ones, on the non-sterile soil, inoculated ones rather grew 4.7% less number of leaves compared to the non-inoculated ones. Whole-soil AMF inoculation of *P. falcatus* seedlings also resulted in a significant 100% increase in relative collar diameter growth rate (Table 4.C).

Discussion

One of the mechanisms to improve dry evergreen Afromontane forests restoration could be the application of AMF biotechnology. Hence, this study on the mycorrhizal responsiveness and growth responses of *C. africana*, *J. procera*, and *P. falcatus*, three of the most important DAF restoration tree species, was timely and relevant. In the previous field experiment, *C. africana* responded negatively to AMF inoculation while, neither *J. procera* nor *P. falcatus* showed a response (Asmelash et al. 2019). Owing to the few growth variables considered and experiment design limitations in the previous experiment, this experiment was crucial. In this experiment, contrary to Asmelash et al. (2019) findings, positive growth responses (i.e., to AMF inoculation and existing potting soil AMF inocula) were recorded for *C. africana*. This finding is also partly against the results by Dobo et al. (2016) that reported little or no AMF inoculation growth effects. However, the difference in the case of Dobo et al. (2016) could most probably relate to seedlings age, i.e., 3 vs. 5 months, which in their case it could be early to detect AMF growth effects.

Similar to the RC levels reported from Ethiopian nurseries (Michelsen 1992; Asmelash et al. 2021), the early successional *C. africana* seedlings were found to have moderate to high RC, while the late-successional *P. falcatus* seedlings had very low RC including on the non-sterile potting soil and also despite receiving whole-soil AMF inoculum. Moreover, similar to the MR trend reported for tropical tree species (Kiers et al. 2000; Zangaro et al. 2003, 2007), we found a high, medium, and low MR of the early, mid, and late-successional tree species, viz., *C. africana*, *J. Procera*, and *P. falcatus* respectively. Hence, it could be the fact that early-successional tree species' success on degraded sites is partly related to the fact that they evolved to be highly arbuscular mycorrhizal. Late-successional tree species, on the other hand, recruited under the shade and on forest soils with better soil attributes, and hence, they may have evolved to be less dependent on AMF at the seedling stage. Hence, in accordance with the optimal partitioning theory that suggests plants' photo-assimilate allocation to organs is proportioned in such a way that whole-plant growth is optimized (Comas et al. 2013), late-successional tree species may allocate more carbohydrate to the shoot/leaf rather than to the root and mycorrhiza to better compete for light. The much higher R:S ratio we recorded for *C. africana* compared to that of *J. procera* and *P. falcatus* (Table 4) is also in alignment with the theory above. However, our results are against the report by Huante et al. (2012) that indicated early successional tropical lowland forest tree species were less responsive to AMF compared to the mid and late successional counterparts. However, it could also be the case that mycorrhizal responsiveness of late-successional tropical tree species increases once the trees have grown well enough and start to partition more carbon to the below-ground biomass (Carrillo-Garcial et al. 1999). Therefore, additional researches are required by using several tree species from the different successional groups to know whether the MR trend observed in this study for *C. africana*, *J. procera*, and *P. falcatus*is due to the tree successional group or taxonomy.

This experiment is different from most AMF inoculation experiments since it was carried out both on sterile and non-sterile growing substrates. For the positively responding tree species, *C. africana*, no inoculation x soil type interaction growth effects were found except SdM. This indicates that AMF inoculation of *C. africana* seedlings has significantly increased most growth variables both on the sterile and non-sterile potting soil. Hence, this may indicate AMF inoculation of *C. africana* could be relevant in a wide range of field conditions. The AMF inoculation x soil type interaction effect on SdM is an indication that there was more SdM due to inoculation on the non-sterile potting soil compared to on the sterile one.

Table 3. Two-way ANOVA results for the effect of AMF inoculation, soil type, and AMF inoculation x soil type interaction on mycorrhizal root colonization and seedling growth

Variables	*C. africana*			*J. procera*			*P. falcatus*		
	Inoculation	**Soil**	**Inoculation x soil**	**Inoculation**	**Soil**	**Inoculation x soil**	**Inoculation**	**Soil**	**Inoculation x soil**
	F	***F***	***F***	***F***	***F***	***F***	***F***	***F***	***F***
AMF root colonization (%)	*42.959****	*148.691****	*18.859****	n.d	n.d	n.d	0.269	0.269	1.301
Leaf number	n.d	n.d	n.d	2.879	5.583*	2.642	2.428	6.159*	7.998**
Leaf area (cm^2)	142.598***	1.000	0.407	n.d	n.d	n.d	n.d	n.d	n.d
Relative leaf number growth rate (per week)	23.203***	0.698	2.608	n.d	n.d	n.d.	n.d	n.d.	n.d.
Relative height growth rate (cm/week)	0.022	0.257	0.010	0.191	0.413	0.009	0.273	0.761	0.794
Relative collar diameter growth rate (mm per week)	6.931*	7.955*	0.944	0.703	0.637	0.229	4.821*	1.772	1.207
Shoot fresh mass (g)	**96.571*****	**0.036**	**1.345**	1.839	3.968	1.617	1.811	0.805	0.013
Shoot dry mass (g)	**120.902*****	**8.479****	**4.913***	1.062	2.569	1.887	0.530	1.993	0.161
Root fresh mass (g)	25.511***	0.087	0.196	0.827	4.44*0	1.382	0.961	5.723*	0.186
Root dry mass (g)	26.821***	0.020	0.082	0.948	2.475	0.275	**0.248**	**3.246**	**0.032**
Plant fresh mass (g)	43.027***	0.413	0.410	4.629	1.429*	1.655	1.576	3.891	0.111
Plant dry mass (g)	59.198***	0.012	0.410	1.081	2.701	1.081	0.012	3.881	0.068
Rooting depth (cm)	1.642	0.425	1.314	0.016	3.176	8.573**	n.d.	n.d.	n.d.
Root to shoot dry mass ratio	0.043	2.709	0.797	1.411	1.547	2.049	1.600	0.867	0.050
Root to plant dry mass ratio	0.080	2.898	1.427	1.949	1.846	2.303	1.283	0.712	0.000

Note: "n.d" not determined. * Significant at $p \leq 0.05$, **significant at $p \leq 0.01$, *** significant at $p \leq 0.001$. Values in bold indicate analysis after log10 transformation. Values in italic indicate significant ($p<0.05$) Levene's statistics.

Table 4. Mean (±S.E) seedling growth comparison for the effects of whole-soil AMF inoculation, soil type, and inoculation x soil type interaction for *C. africana, J. procera, P. falcatus*

Measured variables	Treatments							
	Inoculation		Soil type		Inoculation x soil type interaction			
					S		nS	
	+AMF	-AMF	S	nS	+AMF	-AMF	+AMF	-AMF
C. africana								
AMF root colonization (%)	49.2±2.3 ^{a}	27.4±2.3 ^{b}	18.1±2.3 ^{b}	58.5±2.3 ^{a}	36.2±3.3 ^{a}	0.0 ^{b}	62.2±3.3 ^{a}	54.8±3.3 ^{b}
Leaf area (cm^2)	24.83±1.16 ^{a}	5.2±1.16 ^{b}	1.84±1.16 ^{ns}	14.19±1.16 ^{ns}	26.18±1.6 ^{a}	5.5±1.6 ^{b}	23.4±1.6 ^{a}	4.9±1.6 ^{b}
Relative leaf number growth rate (per week)	0.007±0.004 ^{a}	-0.017±0.004 ^{b}	-0.007±0.004^{ns}	-0.003±0.004^{ns}	0.009±0.005 ^{a}	-0.023±0.005 ^{b}	0.005±0.005 ^{a}	-0.011±0.005 ^{b}
Relative height growth rate (cm/week)	0.02±0.006 $^{n.s}$	0.019±0.006 $^{n.s}$	0.018±0.006 $^{n.s}$	0.022±0.006 $^{n.s}$	0.018±0.008 $^{n.s}$	0.017±0.008 $^{n.s}$	0.023±0.008 $^{n.s}$	0.021±0.008 $^{n.s}$
Relative collar diameter growth rate (mm/week)	0.038±0.004 ^{a}	0.021±0.004 ^{b}	0.021±0.004 ^{b}	0.038±0.004 ^{a}	0.032±0.006 ^{a}	0.009±0.006 ^{b}	0.044±0.006 ^{a}	0.033±0.006 ^{b}
Shoot fresh mass (g)	1.23±0.07 ^{a}	0.45±0.069 ^{b}	0.86±0.07 $^{n.s}$	0.83±0.07 $^{n.s}$	1.29±0.097 ^{a}	0.43±0.097 ^{b}	1.1±0.097 ^{a}	0.48±0.097 ^{b}
Shoot dry mass (g)	0.36±0.02 ^{a}	0.14±0.02 ^{b}	0.23±0.02 ^{b}	0.27±0.02 ^{a}	0.35±0.03 ^{a}	0.11±0.03 ^{b}	0.38±0.03 ^{a}	0.17±0.03 ^{b}
Root fresh mass (g)	1.9±0.15 ^{a}	0.82±0.15 ^{b}	1.33±0.15 $^{n.s}$	1.39±0.15 $^{n.s}$	1.92±0.2 $^{n.s}$	0.74±0.2 $^{n.s}$	1.9±0.2 $^{n.s}$	0.9±0.2 $^{n.s}$
Root dry mass (g)	0.39±0.03 ^{a}	0.15±0.03 ^{b}	0.27±0.03 $^{n.s}$	0.27±0.03 $^{n.s}$	0.4±0.05 $^{n.s}$	0.14±0.05 $^{n.s}$	0.38±0.05 $^{n.s}$	0.15±0.05 $^{n.s}$
Plant fresh mass (g)	3.1±0.2 ^{a}	1.3±0.2 ^{b}	2.22±0.2 $^{n.s}$	2.2±0.2 $^{n.s}$	3.2±0.29 ^{a}	1.2±0.29 ^{b}	3.1±0.29 ^{a}	1.4±0.29 ^{b}
Plant dry mass (g)	0.75±0.04 ^{a}	0.3±0.04 ^{b}	0.5±0.04 $^{n.s}$	0.54±0.04 $^{n.s}$	0.75±0.06 ^{a}	0.26±0.06 ^{b}	0.75±0.06 ^{a}	0.3±0.06 ^{b}
Root to shoot dry mass ratio	1.11±0.117^{ns}	1.076±0.117^{ns}	1.229±0.117^{ns}	0.958±0.117^{ns}	1.173±0.165^{ns}	1.286±0.165^{ns}	1.049±0.165^{ns}	0.867±0.165^{ns}
Root to plant dry mass ratio	0.511±0.025 ^{ns}	0.501±0.025 ^{ns}	0.536±0.025 ^{ns}	0.476±0.025 ^{ns}	0.520±0.035^{ns}	0.552±0.035^{ns}	0.502±0.035^{ns}	0.450±0.035^{ns}
J. procera								
Leaf number	138.75±13.3 $^{n.s}$	106.92±13.3 $^{n.s}$	145±13.3 ^{a}	100.7±13.3 ^{b}	176.2±18.8 $^{n.s}$	113.8±18.8 $^{n.s}$	101.3±18.8 $^{n.s}$	100±18.8 $^{n.s}$
Relative height growth rate (cm/week)	0.06±0.008 $^{n.s}$	0.054±0.008 $^{n.s}$	0.060±0.008 $^{n.s}$	0.053±0.008 $^{n.s}$	0.06±.011 $^{n.s}$	0.058±.011 $^{n.s}$	0.056±.011 $^{n.s}$	0.05±.011 $^{n.s}$
Relative collar diameter growth rate (mm/week)	0.034±.004 $^{n.s}$	0.028±.004 $^{n.s}$	0.033±.004 $^{n.s}$	0.028±.004 $^{n.s}$	0.035±.006 $^{n.s}$	0.033±.006 $^{n.s}$	0.032±.006 $^{n.s}$	0.024±.006 $^{n.s}$
Shoot fresh mass (g)	0.224±0.028 $^{n.s}$	0.171±0.028 $^{n.s}$	0.237±0.028 $^{n.s}$	0.158±0.028 $^{n.s}$	0.288±0.04 $^{n.s}$	0.185±0.04 $^{n.s}$	0.160±0.04 $^{n.s}$	0.157±0.04 $^{n.s}$
Shoot dry mass (g)	0.080±0.01 $^{n.s}$	0.065±0.01 $^{n.s}$	0.084±0.01 $^{n.s}$	0.061±.01$^{n.s}$	0.102±.015 $^{n.s}$	0.067±.015 $^{n.s}$	0.058±.015 $^{n.s}$	0.063±.015 $^{n.s}$
Root fresh mass (g)	0.167±0.03 $^{n.s}$	0.132±0.03 $^{n.s}$	0.19±0.03^{a}	0.11±0.023^{b}	0.23±0.04$^{n.s}$	0.15±0.04$^{n.s}$	0.105±0.04$^{n.s}$	0.115±0.04$^{n.s}$
Root dry mass (g)	0.053±0.008 $^{n.s}$	0.043±0.008 $^{n.s}$	0.057±0.008 $^{n.s}$	0.039±0.008 $^{n.s}$	0.065±0.011 $^{n.s}$	0.048±0.011 $^{n.s}$	0.042±0.011 $^{n.s}$	0.037±0.011
Plant fresh mass (g)	0.391±0.05	0.303±0.05	0.426±0.05^{a}	0.27±0.052^{b}	0.517±0.073 $^{n.s}$	0.335±0.073 $^{n.s}$	0.265±0.073 $^{n.s}$	0.272±0.073 $^{n.s}$
Plant dry mass (g)	0.133±0.018$^{n.s}$	0.107±.018±0.018$^{n.s}$	0.141±0.018$^{n.s}$	0.1±0.018$^{n.s}$	0.167±0.025$^{n.s}$	0.115±0.025$^{n.s}$	0.1±0.025$^{n.s}$	0.1±0.025$^{n.s}$
Rooting depth (cm)	29.733 $^{n.s}$	30.067±1.872 $^{n.s}$	32.258 $^{n.s}$	27.542±1.872 $^{n.s}$	28.217±2.65^{b}	36.3±2.65^{a}	31.25±2.65^{a}	23.833±2.65^{b}
Root to shoot dry mass ratio	0.702±0.07 $^{n.s}$	0.59±0.07 $^{n.s}$	0.705±0.07 $^{n.s}$	0.587±0.07 $^{n.s}$	0.693±0.094 $^{n.s}$	0.716±0.094 $^{n.s\ n.s}$	0.711±0.094 $^{n.s}$	0.464±0.094 $^{n.s}$
Root to plant dry mass ratio	0.407±0.03 $^{n.s}$	0.35±0.03 $^{n.s}$	0.406±0.03 $^{n.s}$	0.35±0.03 $^{n.s}$	0.403±0.04 $^{n.s}$	0.408±0.04 $^{n.s}$	0.41±0.04 $^{n.s}$	0.29±0.04 $^{n.s}$
P. falcatus								
AMF root colonization (%)	5.24±2.8 $^{n.s}$	3.33±2.8 $^{n.s}$	3.33±2.8 $^{n.s}$	5.24±2.8 $^{n.s}$	6.67±4 $^{n.s}$	0.0 $^{n.s}$	4.17±4 $^{n.s}$	6.67±4 $^{n.s}$
Leaf number	41.5±1.02 $^{n.s}$	39.25±1.02 $^{n.s}$	42.167±1.02^{a}	38.58±1.02^{b}	45.33±1.44^{a}	39±1.44^{b}	37.67±1.44^{b}	39.5±1.44^{a}
Relative height growth rate (cm/week)	0.029±0.003 $^{n.s}$	0.031±0.003 $^{n.s}$	0.032±0.003 $^{n.s}$	0.028±.003 $^{n.s}$	0.032±0.004 $^{n.s}$	0.031±0.004 $^{n.s}$	0.026±0.004 $^{n.s}$	0.031±0.004 $^{n.s}$
Relative collar diameter growth rate (mm/week)	0.012±0.002^{a}	0.006±.002±0.002^{b}	0.011±0.002 $^{n.s}$	0.007±0.002 $^{n.s}$	0.016±0.003^{a}	0.006±0.003^{b}	0.009±0.003^{a}	0.005±0.003^{b}
Shoot fresh mass (g)	1.908±0.105 $^{n.s}$	2.108±0.105 $^{n.s}$	2.075±0.105 $^{n.s}$	1.942±0.105 $^{n.s}$	1.967±0.149 $^{n.s}$	2.183±0.149 $^{n.s}$	1.850±0.149 $^{n.s}$	2.033±0.149 $^{n.s}$
Shoot dry mass (g)	0.677±0.04 $^{n.s}$	0.718±0.04 $^{n.s}$	0.737±0.04 $^{n.s}$	0.658±0.04 $^{n.s}$	0.705±.056 $^{n.s}$	0.768±.056 $^{n.s}$	0.648±.056 $^{n.s}$	0.667±.056 $^{n.s}$
Root fresh mass (g)	1.342±0.15 $^{n.s}$	1.55±0.15 $^{n.s}$	1.7±0.15 ^{a}	1.192±0.15^{b}	1.55±0.212 $^{n.s}$	1.85±0.212 $^{n.s}$	1.133±0.212 $^{n.s}$	1.25±0.212 $^{n.s}$
Root dry mass (g)	0.4630.036 $^{n.s}$	0.433±0.0360.036 $^{n.s}$	0.4970.036 $^{n.s}$	0.399±0.036 $^{n.s}$	0.512±0.05 $^{n.s}$	0.482±0.05 $^{n.s}$	0.415±0.05 $^{n.s}$	0.383±0.05 $^{n.s}$
Plant fresh mass (g)	3.25±0.23 $^{n.s}$	3.658±0.23$^{n.s}$	3.775±0.230 $^{n.s}$	3.133±0.23$^{n.s}$	3.517±0.325 $^{n.s}$	4.033±0.325 $^{n.s}$	2.983±0.325 $^{n.s}$	3.283±0.325 $^{n.s}$
Plant dry mass (g)	1.14±0.063 $^{n.s}$	1.15±0.063 $^{n.s}$	1.233±0.063 $^{n.s}$	1.057±0.063 $^{n.s}$	1.217±0.09 $^{n.s}$	1.25±0.09 $^{n.s}$	1.063±0.09 $^{n.s}$	1.05±0.09 $^{n.s}$
Root to shoot dry mass ratio	0.7±0.053 $^{n.s}$	0.605±0.053 $^{n.s}$	0.687±0.053 $^{n.s}$	0.618±0.053 $^{n.s}$	0.743±0.075$^{n.s}$	0.632±0.075$^{n.s}$	0.657±0.075$^{n.s}$	0.579±0.075 $^{n.s}$
Root to plant dry mass ratio	0.403±0.018 $^{n.s}$	0.374±0.018 $^{n.s}$	0.399±0.018 $^{n.s}$	0.378±0.018 $^{n.s}$	0.414±0.025 $^{n.s}$	0.385±0.025 $^{n.s}$	0.392±0.025 $^{n.s}$	0.364±0.025 $^{n.s}$

Note: +AMF: whole-soil AMF inoculated, -AMF: whole-soil AMF not inoculated, S: sterile potting soil, nS: nonsterile potting soil, RC: root AMF colonization, Means containing similar letters (across the row) are not significantly different (p<0.05).

From the previous field experiment (Asmelash et al. 2019), it was found that *J. procera* and *P. falcatus* did not benefit from whole-soil AMF inoculation, although on fertile soil. Similarly, in this study, *J. procera* and *P. falcatus* were found to be less responsive despite grown on degraded soil. These may indicate that instead of going for *J. procera* and *P. falcatus* AMF biotechnology, considering after-planting care as recommended by Negash (2010)could be more beneficial. Moreover, the co-plantation of these tree species with AMF inoculated-highly AM responsive nurse shrub as demonstrated by Barea et al. (2011) and Duponnois et al. (2011)could be the other option. However, it should be noted that we inoculated *P. falcatus* with *J. procera* rhizospheric soil and the low or no inoculation effects may have resulted due to lack of *P. falcatus* specific fungi in the inoculum. According to Wubet et al. (2006) results, co-occurring *J. procera* and *P. falcatus* were found to be colonized by distinct AMF communities and this may also be an indication for their conspecific inoculum requirements. However, *J. procera* did not benefit despite receiving conspecific inoculum and both *J. procera* and *P. falcatus* responded negatively to the existing potting soil AMF inocula. Hence, these may indicate the species have inherent low AMF responsiveness. Moreover, it has been shown that seedlings of tropical tree species may respond positively to a wide range of AMF inocula (Schüßler et al. 2016). Hence, in our case, host AMF inoculum specificity could be less important to the observed low response of the two conifers. For instance, Gavito et al. (2008) have found that, at least at a seedling stage, plant species was a more important determinant of AM response than inoculum type.

In this experiment, control treatment received sterile rhizosphere soil and microbial filtrate. Hence, there is a chance that the AMF inoculation effects observed may be due to the parasitic effect of microbial filtrate (Asmelash et al. 2019). However, the tree species in the study responded similarly both due to inoculation and the existing inocula, i.e., comparisons made between treatments that received microbial filtrate but differ in AMF status. Thus, the MR and AMF growth effects we found for *C. africana*, *J. procera*, and *P. falcatus* were most likely the true responses of the species rather than parasitic effect. The other interesting result found in this study was the negative R:S or R:P of *C. africana* seedlings to both inoculation and existing AMF inoculum despite positive responses for most of the growth variables. A similar result has been reported for tropical early-successional tree species by Zangaro et al. (2007) and in his review, Muleta (2017) has also indicated that AMF inoculation could, in most cases, decrease the R:S of legume plants.

According to Baylis et al. (1963), Podocarpaceae, probability without exception, develop roots with small nodules. In accordance with their assertion, we have also observed several fine root nodules in *P. falcatus* seedlings. These nodules could most probably be inherent and do not develop due to microbial symbiosis (Russell et al. 2002). However, relatively at a later stage of their development (after seedlings grew for 6 months), they could significantly be infected by AMF and they could be an important mechanism to maintain the mycorrhization (Baylis et al. 2003; Russell et al. 2002). Similarly, we have observed that *P. falcatus* seedlings (6 months old) nodules were not colonized by AMF. However, the colonization level could increase with seedlings age which we were not able to determine in this study.

We carried out this experiment to answer three basic questions. Firstly, we wanted to know if the simple technique of whole-soil AMF inoculation of *C. africana*, *J. procera*, and *P. falcus falcatus*, important DAF restoration tree species, could improve their growth. We also wanted to know if inoculation was effective on non-sterile potting soil which resembles field soil conditions. Thirdly, we wanted to compare the mycorrhizal responsiveness of the three tree species and wanted to infer if the tree successional group could be an important factor for mycorrhizal responsiveness, particularly at the seedling stage.

This experiment was able to successfully answer the above research questions. According to our results, whole-soil AMF inoculation was found to be an effective technique. However, it was effective only to the early-successional tree species *C. africana* and not to the mid and late-successional tree species *J. procera* and *P. falcatus*. The fact that *C. africana* responded significantly both on sterile and non-sterile potting soil may indicate its responsiveness under field conditions. In case of *J. procera* and *P. falcatus*, after-planting care could be the best way of ensuring seedlings' field growth, survival, and establishment. In the future massive AMF inoculation experiments in which several DAF tree species from the different successional groups are included should be carried out to know if the tree successional group was an important predictor of tree seedlings'AMF inoculation requirements. These experiments could also investigate the effects of seedling age and time of inoculation.

ACKNOWLEDGEMENTS

AAU & EBI are acknowledged for providing the budget for this experiment. FA would like to thank co-workers at EBI forest gene bank for their assistance at the lab and mesh-house works. Dr. Abreham Abiyou is also acknowledged for providing freshly collected viable *P. falcatus* seeds.

REFERENCES

Abebe G, Mosandl R, El Kateb H, Fetene M. 2010. Restoration of degraded secondary forest with native species: a case study in the highland of Ethiopia. Scand J For Res 25 (S8): 86-91. DOI: 10.1080/02827581.2010.485769.

Aerts R, Negussie N, Maes W, November E, Hermy M, Muys, B. 2007. Restoration of dry Afromontane forest using pioneer shrubs as nurse-plants for *Olea europaea* ssp. *cuspidata*. Restor Ecol 15 (1): 129-138. DOI: 10.1111/j.1526-100X.2006.00197.x.

Asmelash F, Bekele T, Birhane E. 2016. The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. Front Microbiol 7, 1095. DOI: 10.3389/fmicb.2016.01095.

Asmelash F, Bekele T, Belay, Z. 2019. Comparative field survival and growth of selected Ethiopian native tree species and the effect of

whole soil AMF inoculation. J Hort For 11 (2): 19-31. DOI: 10.5897/JHF2018.0573.

Asmelash, F, Bekele, T, Kebede, F, Belay, Z. 2021. The arbuscular mycorrhizal fungi status of selected tree nurseries in the Ethiopian highlands. J For Res 32 (3): 1189-1201. DOI: 10.1007/s11676-020-01169-9.

Barea JM, Palenzuela J, Cornejo P, Sánchez-Castro I, Navarro-Fernández C, Lopéz-García A, Estrada B, Azcón R, Ferrol N, Azcón-Aguilar C. 2011. Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. J Ari Env 75 (12): 1292-1301. DOI: 10.1016/j.jaridenv.2011.06.001.

Baylis GTS, McNabb RFR, Morrison TM. 1963. The mycorrhizal nodules of Podocarps. Trans Brit Mycol Soc 46 (3): 378-384. DOI: 10.1016/S0007-1536(63)80031-5

Bishaw B. 2001. Deforestation and land degradation in the Ethiopian highlands: A strategy for physical recovery. Northeast Afr Stud 8: 7-26. DOI: 10.1353/nas.2005.0014.

Carrillo-Garcial A, Leon de la Luz J-L, Bashan Y, Bethlenfalvay GJ. 1999. Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran desert. Restor Ecol 7 (4): 321-235. DOI: 10.1046/j.1526-100X.1999.72027.x.

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA. 2013. Root traits contributing to plant productivity under drought. Front Plan Sci 4: 442. DOI: 10.3389/fpls.2013.00442.

Dalling JW, Heineman K, González G, Ostertag R. 2016. Geographic, environmental and biotic sources of variation in the nutrient relations of tropical montane forests. J. Trop Ecol 32 (05): 368-383. DOI: 10.1017/S0266467415000619.

Dobo B, Asefa F, Asfaw Z. 2016. Response of selected perennial crops and trees to AMF inoculation and NPK application in pot culture. Acta J Biotech 4: 325-336.

Duponnois R, Ouahmane L, Kane A, Thioulouse J, Hafidi M, Boumezzough A, Prin Y, Baudoin, E, Galiana A, Dreyfus B. 2011. Nurse shrubs increased the early growth of Cupressus seedlings by enhancing belowground mutualism and soil microbial activity. Soil Biol Biochem 43 (10): 2160-2168. DOI: 10.1016/j.soilbio.2011.06.020.

Friis I. 1992. Forests and forest trees of North-East Tropical Africa. Kew Bulletin Additional Series XV. HMSO. London.

Friis I, Demissew, D, Breugel PV. 2010. Atlas of the potential vegetation of Ethiopia. The Royal Danish Academy of Sciences, Biol Skrif 58, Denmark.

Gavito ME, Perez-Castillo D, Gonzalez-Monterrubio C, Vieyra-Hernandez MT, Martínez-Trujillo M. 2008. High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land-use types in a tropical dry ecosystem. Mycorrhiza 19 (1): 47-60. DOI: 10.1007/s00572-008-0203-4.

Giovanetti M, Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84 (3): 489-500. DOI: 10.1111/j.1469-8137.1980.tb04556.x.

Gutjahr C. 2014. Phytohormone signalling in arbuscular mycorrhiza development. Curr Opin Plant Biol 20: 26-38. DOI: 10.1016/j.pbi.2014.04.003.

Hart MM, Reader RJ. 2004. Do arbuscular mycorrhizal fungi recover from soil disturbance differently? Trop Ecol 45 (1): 97-111.

Huante P, Ceccon E, Orozco-Segovia A, Sánchez-Coronado ME, Acosta I, Rincón, E. 2012. The role of arbuscular mycorrhizal fungi on the early stage restoration of seasonally dry tropical rain forest in Chamela, Mexico. Rev Árvore 36 (2): 279-289. DOI: 10.1590/S0100-67622012000200009.

Hunt R. 1990. Basic Growth Analysis: Plant Growth Analysis for Beginners. Unwin Hyman Ltd, UK.

Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza 17 (2): 75-91. DOI: 10.1007/s00572-006-0094-1.

Kiers ET, Lovelock CE, Krueger EL, Herre EA. 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecol. Lett 3 (2): 106-113. DOI: 10.1046/j.1461-0248.2000.00126.x.

Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 82 (8): 1016-1045. DOI: 10.1139/b04-060.

Lemenih M, Teketay, D. 2004. Restoration of native forest flora in the degraded highlands of Ethiopia: constraints and opportunities. SINET: Ethiopian J Sci 27 (1): 75-90. DOI: 10.4314/sinet.v27i1.18225.

Michelsen A. 1992. Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. For Ecol Manag 48 (3-4): 335-344. DOI: 10.1016/0378-1127(92)90154-2.

Muleta D. 2017. Legume response to arbuscular Mycorrhizal fungi inoculation in sustainable agriculture. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for Legume Improvement, 2nd ed. Springer International Publishing, Gewerbestrasse. DOI: 10.1007/978-3-319-59174-2_10.

Negash L. 2010. A Selection of Ethiopia's Indigenous Trees: Biology, Uses and Propagation Techniques. Addis Ababa University Press, Addis Ababa.

Onguene NA, Kuyper TW. 2005. Growth response of three native timber species to soils with different arbuscular mycorrhizal inoculums potentials in South Cameroon Indigenous inoculum and effect of addition of grass inoculums. For Ecol Manag 210 (1-3): 283-290. DOI: 10.1016/j.foreco.2005.02.038.

Ouahmane L, Hafidi M, Kisa M, Boumezzough A, Thoulouse J, Duponnois R. 2006. Lavandula species as accompanying plants in Cupressus replanting strategies: effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. Appl Soil Ecol 35 (2-3): 190-199. DOI: 10.1016/j.apsoil.2006.02.002.

Pandey SK, Singh H. 2011. A simple, cost-effective method for leaf area estimation. J Bot 3: 1-6. DOI: 10.1155/2011/658240.

Pánková H, Münzbergová Z, Rydlová J, Vosátka M. 2014. Co-adaptation of plants and communities of arbuscular mycorrhizal fungi to their soil conditions. Folia Geobotanica 49 (4): 521-540. DOI: 10.1007/s12224-013-9183-z.

Rowe HI, Brown CY, Claassen VP. 2007. Comparisons of Mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restor Ecol 15 (1): 44-52. DOI: 10.1111/j.1526-100X.2006.00188.x.

Russell AJ, Bidartondo MI, Butterfield BG. 2002. The root nodules of the Podocarpaceae harbor arbuscular mycorrhizal fungi. New Phytol 156 (2): 283-295. DOI: 10.1046/j.1469-8137.2002.00504.x.

Schüßler A, Krüger C, Urgiles N. 2016. Phylogenetically diverse AM fungi from Ecuador strongly improve seedling growth of native potential crop trees. Mycor 26 (3): 199-207. DOI: 10.1007/s00572-015-0659-y.

Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis, 3rdedn. Academic Press, Cambridge.

Strassburg BBN, Iribarrem A, Beyer HL, Cordeiro CL, Crouzeilles R, Jakovac CC, Junqueira AB, et al. 2020. Global priority areas for ecosystem restoration. Nature 586 (7831): 724-729. DOI: 10.1038/s41586-020-2784-9.

Teketay D. 1997. Seedling populations and regeneration of woody species in dry Afromontane forests of Ethiopia. For Ecol Manag 98 (2): 149-162. DOI: 10.1016/S0378-1127(97)00078-9.

Verbruggen E, van der Heijden MGA, Rillig MC, Kiers ET. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197 (4): 1004-1109. DOI: 10.1111/j.1469-8137.2012.04348.x.

Vierheilig H, Coughlan AP, Wyss U, Piche Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Env. Microbiol 64 (12): 5004-5007. DOI: 10.1128/AEM.64.12.5004-5007.1998.

Wubet T, Weiß M, Kottke I, Oberwinkler F. 2006. Two threatened coexisting indigenous conifer species in the dry Afromontane forests of Ethiopia are associated with distinct arbuscular mycorrhizal communities. Botany 84 (10): 1617-1627. DOI: 10.1139/b06-121.

Yirdaw E, Leinonen K. 2002. Seed germination responses of four Afromontane tree species to red/far-red ratio and temperature. For Ecol Manag 168 (1-3): 53-61. DOI: 10.1016/S0378-1127(01)00719-8.

Zangaro W, Nishidate FR, Vandresen J, Andrade G, Nogueira MN. 2007. Root mycorrhizal colonization and plant responsiveness are related to root plasticity, soil fertility and successional status of native woody species in southern Brazil. J Trop Ecol 23 (1): 53-62. DOI: 10.1017/S0266467406003713.

Zangaro W, Nisizaki SMA, Domingos JCB, Nakano EM. 2003. Mycorrhizal response and successional status in 80 woody species from South Brazil. J Trop Ecol 19: 315-324. DOI: 10.1017/S0266467403003341.

Zarik L, Meddich A, Hijri M, Hafidi M, Ouhammoua A, Ouahmane L, Duponnois R, Boumezzough, A. 2016. Use of arbuscular mycorrhizal fungi to improve the drought tolerance of *Cupressus atlantica* G. C R Biol 339 (5-6): 185-196. DOI: 10.1016/j.crvi.2016.04.009.