Vol. 11(2), pp. 19-31, February 2019
DOI: 10.5897/JHF2018.0573
Article Number: AD04A2860071
ISSN 2006-9782

http://www.academicjournals.org/JHF

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Journal of Horticulture and Forestry

Full Length Research Paper

Comparative field survival and growth of selected Ethiopian native tree species and the effect of whole soil arbuscular mycorrhizal fungi inoculation

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Received 12 December, 2018; Accepted 7 January 2019

Tree species selection and seedlings lack of infective arbuscular mycorrhizal fungi (AMF) could potentially contribute to low forest restoration success in Ethiopia. Hence, we evaluated the comparative field performance of *Cordia africana (C. africana)*, *Juniperus procera (J. procera)*, and *Podocarpus falcatus* (*P. falcatus)* seedlings and the effect of whole soil AMF inoculation. Seedlings prepared using the existing practice were planted on a field at Chancho, Central Ethiopia. Seedlings were inoculated with six types of whole soil AMF inoculums, no inoculation being the control. Seedlings survival was determined at the end of the 11th month; relative growth rate in collar diameter (RGR-CD) and height (RGR-H) was determined at the 1st, 2nd, 5th, 8th, and 11th months. We found out that the plantation site were very fertile with available phosphorus (Av P) =103.16 mg/Kg; total nitrogen (TN) = 0.376%; organic carbon (OC) = 3.12%; AMF spore abundance = 6.91±2.00 g^{-1}. None of the *P. falcatus and C. africana* seedlings survived while 25% of *J. procera* seedlings survived. AMF inoculation did not have statistically significant effect on RGR-CD and RGR-H but conspecific inoculum resulted in significantly lower RGR-H of *C. africana* at the 2nd month, indicating the probability of species specific effect of AMF inoculation.

Key words: Arbuscular mycorrhizal fungi (AMF), Chancho, forest restoration, relative growth rate (RGR), seedlings survival.

INTRODUCTION

Ethiopia has put a plan of restoring 15 million hectares of its degraded land by 2025 (UNDP, 2016). Hence, billions of tree seedlings, including native trees are being planted every year. Meanwhile, an estimated 300 million $USD is being invested each year in seedlings' preparation and labour. Hence, reduced level of seedlings survival and establishment which is a reality in Ethiopia (Abebe et al., 2011; Muluneh, 2017), is not only a bottleneck to the realization of the national restoration target but also translates to the loss of money in tens, if not hundreds of millions of US dollar annually. Therefore, improving field survival and establishment rates of planted seedlings is

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probably the single most important intervention towards the realization of Ethiopia's national restoration target while it is also critically important to reduce the restoration cost. The most important reasons identified for the observed and documented poor field survival and establishment of Ethiopian native trees are; inappropriate species selection, lack of aftercare, and moisture and nutrient stress at the planting site (Negash, 2010; Reubens et al., 2011; Mahari, 2014). Meanwhile, to significantly improve tree seedlings field survival and establishment, both the identification of suitable tree species for a particular planting site and providing the necessary aftercare are indispensible. Furthermore, searching for technologies that enhance tree seedlings moisture and nutrient relation is also important. One potential such technology could be arbuscular mycorrhizal fungi (AMF) inoculation (Asmelash et al., 2016).

The potential role of AMF inoculation for ecological restoration has been well recognized even before restoration ecology emerged as a scientific field of study (Janos, 1980). AMF inoculation, among others, can significantly improve tree seedlings nutrients and water relation to potentially improve their field survival, establishment and growth thereby improving forest restoration success (Asmelash et al., 2016; Neuenkamp et al., 2018). AMF inoculums either comprise single species or are consortia of species or are whole soil inoculums of which, the latter is known to have much better positive effect (Hoeksema et al., 2010). Similarly, restoration ecologists recommend whole soil AMF inoculation hence recognize the mixing of a handful of soil from the target forest to the planting holes to be the simplest and cheapest way to initiate AMF inoculation (Elliott et al., 2013). Likewise, in Ethiopian nurseries, mixing forest soil in nursery pot soil is a common nursery procedure. However, quite commonly, forest soil inoculation may be skipped during the nursery management. When not skipped, forest soil is commonly stored for many months in an open air condition which may reduce the infectivity of fungi. Hence, due to these and other possible reasons, tree seedlings raised in Ethiopian nurseries could be withlow infective AMF inoculum (Michelsen, 1992).

AMF are ubiquitous, found almost in every soil and AMF species are assumed to be generalists able to form symbiotic relationship with all plants that are arbuscular mycorrhizal (Abbott and Robson, 1991; Brundrett and Abbott, 2002). However, similar to other reports elsewhere, native trees of Ethiopia that do co-occur (Wubet et al., 2006) and conspecific adult and seedlings of these native trees (Wubet et al., 2009) were observed to associate with distinct AMF communities possibly indicating that tree seedlings could have inoculum preference. Therefore, for his experiment, inoculums prepared from the rhizosphere of adult and seedlings/saplings of the corresponding tree species found in nearby forest were used. Furthermore, autochthonous inoculum prepared from the top soil of the planting site was also prepared. Onguene and Kuyper (2005) argue that, for better growth effect on seedlings, autochthonous or planting site adapted inocula are more appropriate than allochthonous inocula prepared from forests.

The main objectives of this research were to determine the comparative suitability of the Ethiopian native trees; *Cordia Africana (C. africana)*, *Juniperus procera (J. procera)*, and *Podocarpus falcatus* (*P. falcatus)* for forest restoration in the Chancho environs, central highland of Ethiopia and investigate the possible effect of quality whole soil AMF inoculation in improving the field survival and growth of seedlings of these tree species that are raised through the existing nursery practices. While *J. procera* and *P. falcatus* are chosen because they are typical tree species of the dry afromontane climax forests, *C. africana* was chosen due mainly to the fact that it is one of the most commonly raised tree species in the Ethiopian highland nurseries. The other objective of this research is to investigate the effect of AMF inoculum source (forest Vs. planting site, adult Vs. seedlings, conspecific Vs non-conspesfic) on the seedlings survival, RGR-CD, and RGR-H.

This research was initiated due to the fact that there are very few tree seedlings field survival and establishment experiments in Ethiopia. Previously, Abebe et al. (2011) conducted comparative field survival and establishment experiment on *C. africana*, *P. falcatus*, and *J. procera* tree seedlings prepared through the existing nursery practices and planted in the gaps of Munesa forest, central-east highland of Ethiopia and reported that, after two years, the field survival/ establishment rates were 23, 46, and 76% respectively. Furthermore, outfield AMF inoculation experiments in relation to forest restoration are very scarce not only in Ethiopia but also globally (Asmelash et al., 2016; Duponnois et al., 2016) making this experiment more relevant and timely. This field experiment was conducted on a fallowed farm land near Chancho town and the forest used as inoculum source was Menagesha-suba forest located some 40 km away from the planting site.

MATERIALS AND METHODS

Study species

The species selected for this study are *C. africana, J. procera,* and *P. falcatus*. These species are native to Ethiopia and provide numerous economic and ecological benefits (Negash, 2010). C. africana is a deciduous broad leaved tree which grows up to the height of 10-15 m; rarely reaching 25 m while J. procera and P. falcatus are both evergreen conifer tree species that grow up to the heights of 35-40 m and 30-35 m respectively (Friis, 1992). C. aficana is an early-successional tree (Friss, 1992; Yirdaw et al., 2002) that has a wide spread distribution in Ethiopia growing in the areas with the altitudinal ranges of 550-2600 m.a.s.l and mean annual rainfall of 700-2000 mm (Friis, 1992). *J. procera* and *P. falcatus* are both dioecious species (Negash, 2010) that can best

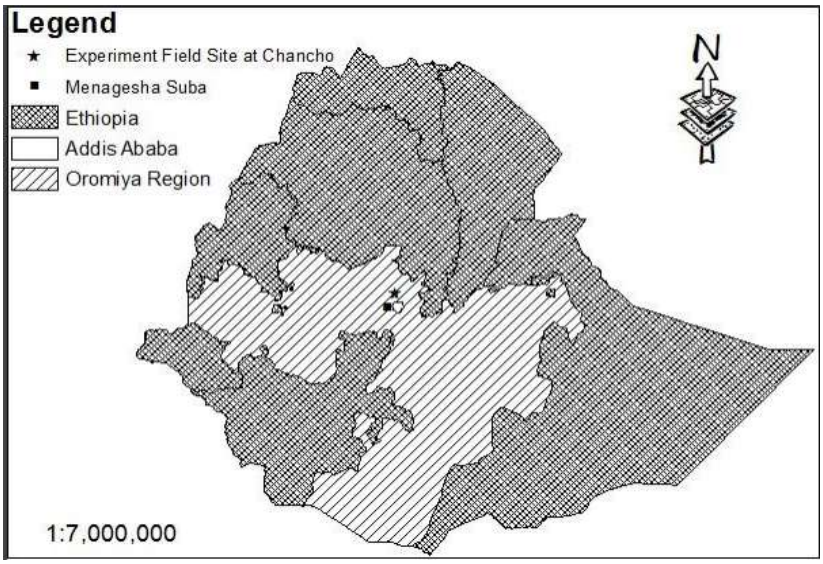

Figure 1. Map of the study area.

be described as a mid-successional and late-successional, respectively (Abebe et al., 2010; Teketay, 1997).

J. procera is a characteristic tree species of the dry evergreen afromontane forests of Ethiopia and grows in the areas with altitudinal ranges of 1100-3500 m.a.s.l and mean annual rainfall of 500-1000 mm (Friis, 1992). Similarly, although *P. falcatus* can be found in the moist afromontane forests of Ethiopia, it is basically a characteristic species of the dry evergreen afromonate forests and grows in areas with altitudinal ranges of 1550-2800 m.a.s.l. receiving mean annual rainfall of 1000-2000 mm (Friis, 1992). *C. africana* is animal and bird pollinated tree species that is relatively fast growing on moderate conditions but soil nutrient deficiency mainly P and N limits its growth potential (Negash, 2010). Both *J. procera* and *P. falcatus,* on the other hand, are wind pollinated and hardly establish and grow slowly on moisture and nutrient limited sites (Negash, 2010). Owing to illicit cutting, habitat fragmentation, and loss, both *J. procera* and *P. falcatus* are conservation priority species whose genetic resource of the former is recognized by FAO as being severely degraded and for that of the latter, only less than one per cent of its previous populations is remaining (Negash, 1995). Based on previous reports, *C. africana* (Birhane et al., 2010; Chanie and Assefa, 2013), *J. procera* and *P. falcatus* (Wubet et al., 2003) were already known to be arbuscular mycorrhizal.

Description of the study area

This experiment was carried out on a fallowed farmland at the fringe of Chancho town at an altitude of 2594 m.a.s.l. and located at 09°17’31.9’’N, 038°44’45.1’’E, central Ethiopia (Figure 1). Chanco is found about 40 km north of Addis Ababa along the main Addis Ababa-Bahirdar road. The soil of the area is described as Eurtic vertisol (Berhanu et al., 2013).

Chancho is thought to be once covered with diverse multi-layered dry evergreen montane forests that were dominated by tree species such as *J. procera, P. falcatus* and *Olea europaea* ssp. Cuspidata (Yirdaw and Luukkanen, 2003). Currently, however, there are no natural forests remaining and the land cover predominantly comprises Pennisetum grassland. Yirdaw and Luukkanen (2003) spotted the only place where natural forest was found to be at a churchyard. Owing to the fact that Pennisetum grassland is a major land use, dairy farming is the main activity of the farmers in addition to growing cereals such as barley and oat, pulses mainly beans and tuber crop potato. The Pennisetum grass serves the local people as an important source of income. Hence, at the end of the rainy season, it is cut and piled up to be later sold as far as Addis Ababa and Mojo.

Menagesha-Suba state forest is found in Southwest of the experiment field (42 km aerial distance) and southwest of Addis Ababa (30 km road distance). This is a dry afromontane forest situated near the experiment field site and hence, was used as source of AMF inocula (Figure 1). Menagesha-Suba is located in two districts of the Oromiya region namely; Sebeta-Awas and Welmera. The Menagesha-Suba forest currently comprises a 2667.6 ha of natural forest and 2783.7 ha of plantation forest. Menagesh suba forest was once a grass land or farmland before the then king Zeray yacob (1597-1603) ordered its reforestation by using *J. procera* seeds originating from Wof-Washa forest (Alemayehu et al., 2009; Sertse et al., 2011). Now the forest has matured into, according to Friis et al. (2010), a kind of undifferentiated afromontane forest with canopy of J. *procera* and *P. falcatus*. The prominent species, *J. procera* and *P. falcatus* have been either deliberately planted or regenerated naturally very long while only a small patch of matured *C. africana* stand exists which was planted in recent years. The forest has, as the name implies, two major forest parts; the Suba side forest and the menagesha side forest. Meanwhile, in this study, from the suba side of the forest, rhizospheric soils from the adult and seedlings/saplings of *J. procera* and *P. falcatus* were collected while for *C. africana*, owing to the lack of seedlings/saplings, it was only from the adult individuals that rhizosphere soil was collected. The collected rhizospheric soil was later used to produce whole soil AMF inoculums. Similarly top soil from farm within the experiment field was collected and was used to prepare planting site adapted inoculum.

The experiment field site at Chancho has mean annual temperature of 15.6°C and mean annual rainfall of 1225.7 mm with the dry season spanning from November to mid-February and the main rainy season lasting from end of May to September making the months November and December both the driest and the coldest months (Figure 2).

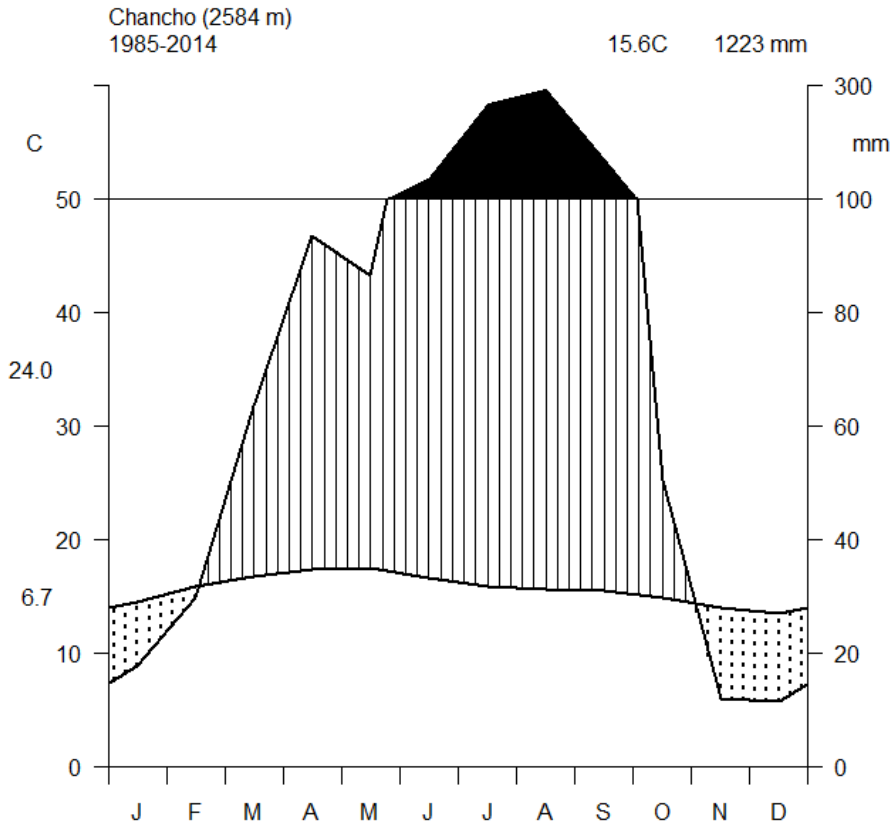

Figure 2. Climate diagram of Chancho (Based on the data from https://climatecharts.net/)

Inoculum preparation

According to Brundrett et al. (1996) and International Culture Collection of (Vesicular) arbuscular mycorrhizal fungi (INVAM), trap cultures generally contain many more viable spores than the field soil they are derived from. Hence, in this experiment, trap culture inoculum was used instead of direct rhizosphere soil inoculum such that inoculums may contain more healthy and infective spores. Most importantly, trap culture and not rhizosphere soil was used in order to mimic the conventional inoculum production method.

Trap culture inoculums were prepared following the protocol described by INVAM (https://invam.wvu.edu/). Hence, rhizospheric soil was collected from Menagesha-suba state forest in March 2016. Rhizospheric soil was collected from adult and seedling/ saplings of the study tree species. For the seedlings/saplings, rhizospheric soil was obtained after uprooting. For the adult individuals, soil surrounding lateral roots, when applicable with fine roots, was collected. *J. procera* and *P. falcatus* ,both being dioecious species, the required small amount of rhizospheric soil was collected from more than one individual that are found at nine different locations such that both sexes may likely be captured. The collected rhizospheric soil was immediately brought to the Addis Ababa University (AAU) in plastic bags. In similar fashion, top soil from the experiment field site was collected and brought to AAU.

The collected rhizospheric soil and the top soil were thoroughly mixed and allowed to air dry for few hours. Then, the air dried soil was mixed with washed and autoclaved river sand (1:1 by volume). Sand was autoclaved twice in a 24 h interval for 30 min each. Then, on each pot containing the trap soil, 100 Sudan grass (*Sorghum x drummondii*) seeds, obtained from international livestock research institute (ILRI), Addis Ababa, were sown after being disinfected by rinsing them with 10% hypo chlorate for five minutes. The Sudan grass was grown in the greenhouse at the AAU for four months. Watering was done to the field capacity every day for 14 weeks. When sign of rust was observed, two tea spoons diammonuim phosphate (DAP) were applied once as recommended by Miyasaka et al. (2003). At the end of the 14th month, watering was halted. The pot culture was then brought to the lab and allowed to dry slowly for two weeks under shade. Finally, the shoots were cut down and the soil containing the roots was dumped on plastic bags to serve as whole soil AMF inoculum. This way, the inoculum types prepared were; 1) inoculum from *C. africana* adult (CoA), 2) inoculum from *J. procera* adult (JuA), 3) inoculum from *J. procera* seedlings/saplings (JuS), 4) inoculum from *P. falcatus* adult (PoA), 5) inoculum from *P. falcatus* seedlings/saplings (PoS), and 6) inoculum from the experiment site/planting site adapted inoculum (PSA). The 7th inoculum was no inoculation (Control).

Relative growth rate and RGR-CD and RGR-H determination

Relative growth rate (RGR) expresses growth in terms of a rate of increase in size per unit of size (Hunt, 1990). Analyzing RGR of seedlings is one method used to compare growth differences that arise from experimental treatments and it is popular with many forest researchers (Rees et al., 2010). It is also believed to be one of the most ecologically significant and useful indices of plant growth specially when comparing seedlings that differ in initial size (Hunt, 1990; Rees et al., 2010; Pommerening and Muszta, 2015). Applications of RGR is mainly to dry weight but also includes other

Figure 3. A view of the experiment field with fence around.

variables such as, leaf area, stem volume or diameter, basal area, and stem diameter (Pommerening and Muszta, 2015). Likewise, in this field experiment, RGR of seedlings collar diameter and height is used to compare effect of treatment factors. Low relative growth rates for extended periods of time are good indicators of imminent death (Pommerening and Muszta, 2015) while negative RGRs are relative decay rates (Hunt, 1990).Collar diameter and height of seedlings were measured every month using digital calliper and ruler respectively. RGR-CD and RGR-H was computed following Saveyn et al. (2010). Hence, RGR-CD = (ΔD/t/Di) x 100 or RGR-H= (ΔH/t/Hi) x 100, where ΔD is change in seedlings collar diameter , ΔH is change in seedlings height, "t" is time in days, Di is initial collar diameter, and Hi is initial seedling height. RGR-CD and RGR-H were computed for the 1st, 2nd, 5th, 8th, and when applicable, 11th months (August 20-September 20, September 20-October 20, October 20-January 20, January 20-April 20, and April 20-July 20 respectively) deliberately to compare the RGR of the tree species and AMF inoculation effect at different soil moisture conditions. 1st and 2nd months represent moist periods with 1st month being the moistest, 5th month represents the onset and elapse of both moisture and cold stress period compared with 2nd month. 8th month represents drought period while 11th month represents the onset of wet period when some rain showers appear after long dry season (Figure 2). Meanwhile, Di is collar diameters measured at plantation, at the 1st month, 2nd month, 5th month, and 8th month for RGR-CD determined at the 1st month, 2nd month, 5th month, 8th month, and 11th month respectively while Hi is height measured at plantation, at the 1st month, 2nd month, 5th month, and 8th month for RGR-H determined at the 1st month, 2nd month, 5th month, 8th month, and 11th month respectively.

Experiment design

The experiment was carried out from August 20, 2016 until July 28, 2017 for 11 months. The experiment was laid in split plot design. Tree species were the main plot and AMF inoculums sub plots. Split plot design was chosen so that management of the experiment would be much simpler. Each plot consisted of 4 seedlings with 1m x 1m spacing and spacing between plots was 1.5 m. Sub plots assumed RCBD design where randomization was done by using the random number table and basically for each tree species the randomized arrangement of treatments was made similar.

According to Miyasaka et al. (2003) and Onguene and Kuyper (2005) inoculums of 50 g per kilogram of soil is good enough to have effective inoculation. If it is more than 50 g, it may serve as a source of carbon and nutrients and therefore may not be appropriate (Birhane et al., 2015). Therefore, during seedlings planting on the experiment field site on August 20, 2016, from the six inoculum types prepared, an estimated 50 g of inoculum (soil with infected Sorghum roots) was applied to serve as whole soil AMF inoculum. The control received no inoculum.

The seedlings used in this experiment were obtained from Susuni Nursery, Addis Ababa situated at an altitude of 2515 m.a.s.l. The seedlings were raised using the existing nursery protocol whereby pot soil was prepared by mixing reddish clay top soil: compost: sand (3:2:1). The seedlings used in this study had initial mean collar diameters of 6.24± 0.12, 4.19 ± 0.8, and 3.2 ± 06 mm and initial mean heights of 26.54 ± 0.41, 28.18 ± 0.31, and 11.55 ± 0.27 cm respectively for *C. africana, J. procera,* and *P. falcatus*. In order to halt or significantly minimize herbivory, the experiment field was fenced before the seedlings were planted (Figure 3). At the end of

Table 1. Soil characteristics of the field site with ±SE when available.

Soil cover	Texture/clay content	pH		EC	OC (%)	Ave P (mg/kg)	TN (%)	CEC (meq/100 g)	Na (cmol/kg)	K (cmol/kg)	Spore abundance (g^{-1})
		H_2O	KCl								
100%	Clay loam/42%	6.13±0.03	5.0	0.133 ds/m	3.12	103.16	0.376	49.91	1.04	1.13	6.91±2.00

the first month *P. falcatus* seedlings were observed to be fully engulfed by grass weeds. Therefore, a complete hand weeding was carried out for P. falcatus plots to free the seedlings. Throughout the experiment period no other management activity was carried out.

The month at which RGR was computed denoted as "month" hereafter is the third factor in this experiment. Therefore, this experiment is a three factorial design with tree species (*C. africana, J. procera, and P. falcatus*), seven AMF inoculums (Control, CoA, JuA, JuS, PoA, PoS, and PSA), and 5 months (1st month, 2nd month, 5th month, 8th month, and 11th month).

Soil characterization

A composite 1 kg sample soil was collected from the experiment field site in October 2016 after the onset of the dry season. Selected soil chemical and physical characteristics such as soil texture, soil pH (H_2O), and AMF spore abundance were determined at the Addis Ababa University Ecology and Ecophysiology Laboratory. Soil texture was determined using hydrometer method and using sodium hexametaphosphate as dispersant. pH (H_2O) was measured potentiometrically using a digital pH meter in a 1:5 soil water suspension (Cottenie, 1980). AMF abundance, the number of spores g^{-1} of air dried soil, was determined by using the INVAM protocol after the spores were separated from the soil by the wet sieving and decanting method as illustrated by Brundrett et al. (1996).

Fifty gram soil sample was mixed in a substantial volume of water and thoroughly washed and decanted several times through a series of sieves with 1 mm (to remove large debris), 180 µm (to obtain big spores), 90 µm (to obtain medium sized spores) and 53 µm (to obtain small spores). The contents on 180 µm and 90 µm sieves were washed off with tap water and added with the contents on the 53 µm sieves. Then the soil was washed using tap water and then using wash bottle, it was carefully transferred to test tubes in three replications. The contents on the test tubes were centrifuged for 5 min at 2000 RPM. After having discarded the supernatant, the pellets were re-suspended in 50% sucrose solution, shook very well and then centrifuged for 1 min at 2000 RPM. The supernatant was carefully poured through a 53 µm sieve and carefully washed with water to remove the sucrose. Finally, the supernatant was carefully washed off on to 9 cm diameter plastic Petri dishes by using distilled water and wash bottle. Finally, the spore and microscopic debris suspension was observed under a 2x dissecting microscope and the spores counted. AMF spores were mostly colorful distinctive from the debris and other microbial bodies. Since the diameter of the ocular vision was found to be 9 mm, the total vision to cover the whole Petri dish was determined to be 100. Meanwhile, after taking 40 separate microscopic views per Petri dish, the average was multiplied by 100 to estimate the average spore number per 50 g. This spore number was also finally converted to spore number per g^{-1} of soil just by dividing it by 50. The portion of the soil sample was also sent to the National soil testing center of Ethiopia to determine pH (KCl), electrical conductivity (EC), cation exchange capacity (CEC), plant available phosphorus (Ave P) using Olsen method, total nitrogen (TN), exchangeable bases (Na & K) and organic carbon (OC).

Data analysis

Data were analysed using R software version 3.4.2 (The R Foundation for Statistical Computing Platform, 2017), SYSTAT 13.1 software (systat software inc., USA) and SPSS software version 20 (SPSS 20 software IBM corp., NY., USA). R software was used to construct and plot the climate diagram while statistical analysis was carried out using SYSTAT and SPSS. For the only surviving tree species at the 11th month (*J. procera*), using SPSS, the effect of AMF on the survival rate was determined by non-parametric Kruskal wallis test while parametric one way ANOVA was used to determine AMF inoculation effect on RGR-CD and RGR-H.

To determine the effect of species, inoculation, month, and the interaction on RGR-CD and RGR-H, bootstrap three-way ANOVA using SYSTAT was run owing to the fact that the underlying assumptions to carry out parametric mixed three way ANOVA were not met. For those factors with statistically significant effect on RGR-CD and RGR-H, bootstrap Tukey HSD ($P<0.05$) was computed. Furthermore, for factors with significant two way and three way interaction effect, one way Welch ANOVA and parametric one way ANOVA were computed using SPSS and for factors with significant effect, post-hoc test using Tukey HSD ($P<0.05$) was carried out and its graph plotted using SYATAT. When applicable, mean comparison using Fisher's LSD ($P<0.05$) was also used.

RESULTS

Soil characteristics

The soil of the experiment field was found to be slightly acidic with comparatively high AMF spore abundance. The soil was 100% covered with grasses and herbs. The grass species; Pennisetum sp., Eragrostis sp., *Eleusine floccifolia*, Avena sp. and herbs namely *Carduus schimperi, Conyza steudelli*, *Hellchrysum splendidum, Rumex abyssinicus*, *Trifolium quartinianum* were among the observed plants that occupy the field as weeds at a time or in succession.TN, OC, and most importantly Ave P of the experiment field site indicated that the site was very fertile (Table 1).

Seedlings survival and the effect of AMF inoculation

At the end of the 11th month, none of the *P.*

Table 2. Result of non-parametric Kruskal Wallis test showing that none of the AMF inoculums had statistically significant effect on the survival rate of *J. procera* seedlings at the 11th month.

Statistical parameter	Survival of *J.procera*
Chi-Square	3.146
df	6
P-Value	0.790

P. falcatus or *C. africana* seedlings survived. But for *J. procera*, 28 of the seedlings (25%) survived. Furthermore, non-parametric Kruskal Wallis test indicated that AMF inoculation has no statistically significant effect on the survival rate of *J. procera* seedlings (Table 2).

RGR-CD and the effect of AMF inoculation

Statistically significant variation (P<0.001) in RGR-CD was found between the tree species. Month was also found to have statistically significant effect (P<0.001) on RGR-CD. However, AMF inoculation was found to have no statistically significant effect on RGR-CD. Significant two way species x month interaction effect (P<0.001) was also present but no species x inoculation or species x inoculation x month interaction effect was found (Table 3). Interaction effect between species and month indicates that the comparative RGR-CD of tree species varies with month and/or the effect month has on RGR-CD varies with tree species (Figure 4). Furthermore, at the 11th month, for *J. procera*, the only surviving species, AMF inoculation was also found to have no statistically significant effect (F= 0.435) on the RGR-CD (Table 4).

Mean comparison carried out using bootstrap Tukey's HSD (P<0.05) shows that *J. procera* and P. falcatus have no statistically significant mean difference in RGR-CD while *C. africana* had statistically significantly smaller RGR-CD compared to both *J. procera* and *P. falcatus*. The mean comparison also shows that statistically, mean RGR-CD at 1st, 2nd, and 5th month statistically significantly varied (P<0.05) with the order of 1st month > 2nd month > 5th month. 8th month resulted in statistically significant higher (P<0.05) RGR-CD compared with 5th month but had no statistical pairwise difference (P<0.05) with 1st and 2nd months (Figure 4).

The Welch ANOVA result shows that all the three species were responsible for the observed overall statistically significant effect on RGR-CD. Similarly, all the four months were also responsible for the statistically significant variation in RGR-CD (Table 5). In all the months except 2nd month, J. procera seems to have better mean RGR-CD compared with the other two species. At the second month, it was P. falcatus that registered the highest RGR-CD. During the harshest month, at the 5th month, RGR-CD had statistically different mean (P<0.05) in the order of J. procera >P. falcatus > C. africana (Figure 4).

In Figure 4, the specis x month interaction effect is clearly seen. While the overall mean differnce between *J. procera and P. falcatus* was not statistically significant (P<0.05), for the 2nd and 5th month separatly, it was statistically significant (P<0.05) with the order of *P.falcatus* > *J. procera* and *J. procera* >*P. falcatus* respectively.

RGR-H and the effect of AMF inoculation

Statistically significant (P<0.001) variation in RGR-H was found between the study species. Month was also found to have statistically significant effect on RGR-H (P<0.001). However AMF inoculation had no statistically significant effect on RGR-H of all the three tree species for the first 8 months. Tree species x month and species x inoculation x month did show statistically significant interaction effect (P<0.001 and P<0.05 respectively) on RGR-H while no statistically significant interaction effect was found for species x inoculation or month x inoculation (Table 6). The statistically significant interaction effect present between species and month indicates that comparative RGR-H of the three tree species was variable across the months (Figure 5). Furthermore, the interaction species x inoculation x month indicate that inoculation has statistically significant effect on RGR-H at least for a particular month and species (Figure 6). This is supported by the result of parametric one way ANOVA which indicated inoculation effect had significant effect on RGR-H of *C. africana* at the 2nd month (Table 7). For *J. procera*, the only surviving species at the 11th month, AMF inoculation was also found to have no statistically significant effect (F=0.928) on RGR-H (Table 4).

The Welch ANOVA result shows *J. procera* did not have statistically significant variation in RGR-H across the months while the remaining two species namely *P. falcatus* and *C. africana* had statistically significant variation in RGR-H across the months (Table 6). Similarly, at the first month, no statistically significant variation in RGR-H was recorded across the species while for the remaining months, RGR-H did indeed vary across species. For these months with variation, *J. procera* seem to do better except the 2nd month when P. falcatus did grow significantly higher compared with that of *J. procera* and *C. africana* (Figure 5). The overall mean comparison indicates that statistically significant variation (P<0.05) in RGR-H exists between study species with *P. falcatus* > *J. procera* > *C. africana* (Figure 5A). With regards to the month, distinctively different effect was observed for the wet and dry seasons with statistically significant (P<0.05) mean difference observed between the wet (1st and 2nd) and dry (5th and 8th) months (Figure 5-B). The one way parametric ANOVA carried out to determine for which species and at which month did

Table 3. Three way bootstrap ANOVA to identify the effect of tree species, month and AMF inoculation and their interaction on RGR-CD. Bootstrap was for 500 resampling and "***" indicates there was statistically significant effect (p<0.0001).

Source	Type III sum of squares	df	Mean square	F-Ratio	P-value
Spp	4.547	2	2.273	48.070	0.000***
Month	2.504	3	0.835	17.651	0.000***
Inoculation	0.178	6	0.030	0.628	0.708
Spp x Month	3.750	6	0.625	13.215	0.000***
Spp x Inoculation	0.147	12	0.012	0.258	0.994
Month x Inoculation	0.616	18	0.034	0.723	0.786
Spp x Month x Inoculation	1.224	36	0.034	0.719	0.883
Error	11.918	252	0.047		

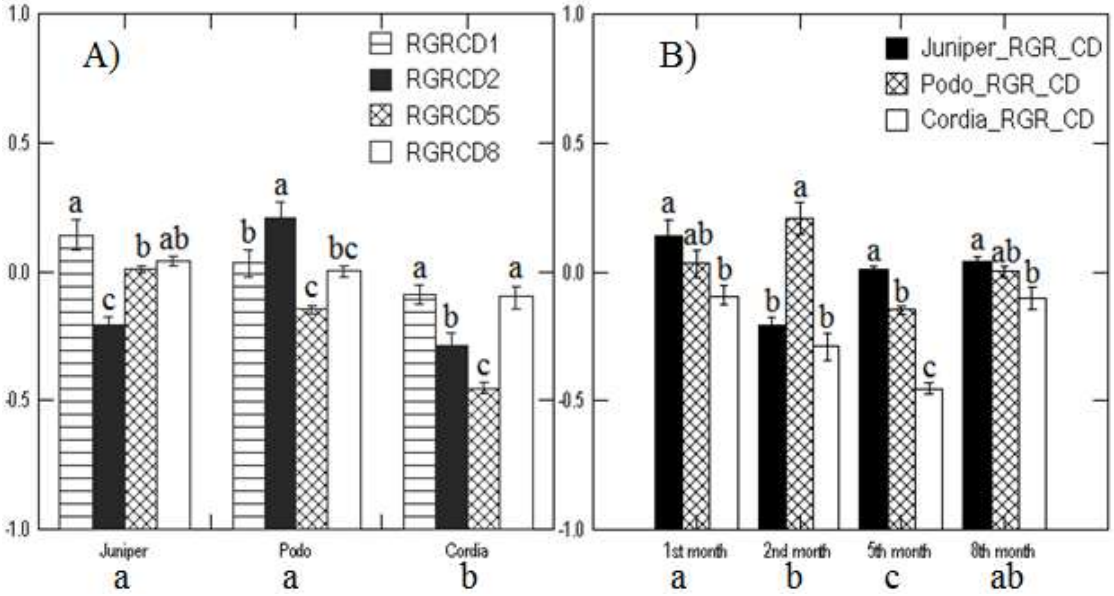

Figure 4. Mean comparison for RGR-CD within species (A) and between species (B) across the months using Tukey HSD (P<0.05). Different letters indicate statistically significant difference in mean RGR-CD and letters below the graph show overall pairwise mean comparison.

Table 4. Parametric one way ANOVA result for AMF inoculation effect on RGR-CD and RGR-H of *J. procera* at the 11th month.

Parameter	Sum of Squares	df	Mean Square	F	P-Value
RGR-CD	0.521	6	0.087	0.435	0.847
RGR-H	0.867	6	0.145	0.928	0.495

whole soil AMFs inoculation have statistically significant effect on RGR-H indicated that it was only for *C. africana* seedlings and at the 2nd month when conditions for plant growth were better that inoculation have statistically significant effect (Table 8). Furthermore, it was possible to determine which inoculum was responsible for the observed significant effect. Hence, it was determined that the conspecific inoculum prepared from adult *C. africana* tree rhizosphere was responsible for the observed effect resulting in statistically significantly low RGR-H compared with the other inoculums and the control (Figure 6).

DISCUSSION

Experiment field soil condition and spore abundance

The experiment field site has good fertility may be due to

Table 5. Welch ANOVA for the effect of species and month on RGR-CD. "***" and "**" indicate there was statistically significant effect (P<0.0001) and (P<0.05) respectively.

Species	RGR-CD			
	Statistic	**df1**	**df2**	**P-Value**
J. procera	17.103	3	53.912	0.000***
P. falcatus	22.016	3	53.650	0.000***
C. africana	31.119	3	56.591	0.000***
Month	**Statistic**	**df1**	**df2**	**P-Value**
1st month	5.807	2	52.521	0.005***
2nd month	22.687	2	50.260	0.000***
5th month	175.866	2	51.406	0.000***
8th month	4.493	2	50.842	0.016**

Table 6. Three way bootstrap ANOVA for species comparative RGR-H, the effect of month, AMF inoculation and their interaction. Bootstrap was done with 500 resampling. "***", "**" indicate statistically significant effect (P<0.001, P<0.05 respectively).

Source	Type III sum of squares	df	Mean Square	F	P-Value
Spp	3.002	2	1.501	38.848	0.000***
Month	2.492	3	0.831	21.499	0.000***
Inoculation	0.373	6	0.062	1.611	0.145
Spp x Month	3.468	6	0.578	14.956	0.000***
Spp x Inoculation	0.626	12	0.052	1.351	0.191
Month x Inoculation	0.917	18	0.051	1.319	0.176
Spp x Month x Inoculation	2.202	36	0.061	1.583	0.024**
Error	9.661	250	0.039		

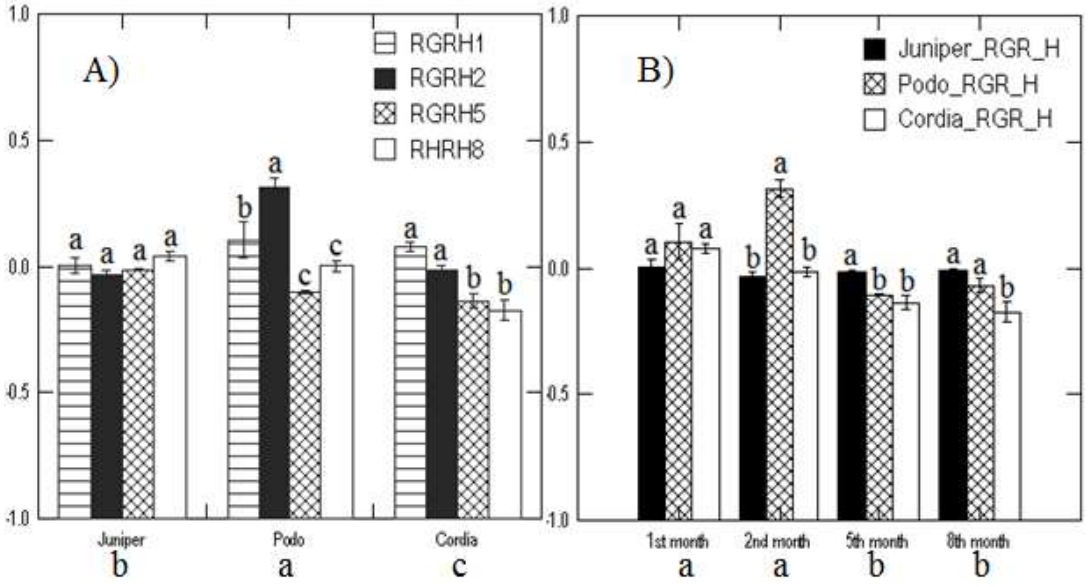

Figure 5. Mean comparison for RGR-H between species and interaction with month (A) and between months and interaction with species (B) using Tukey HSD (P<0.05). Different letters indicate statistically significant difference in mean RGR-H and letters below the graph show overall mean comparison.

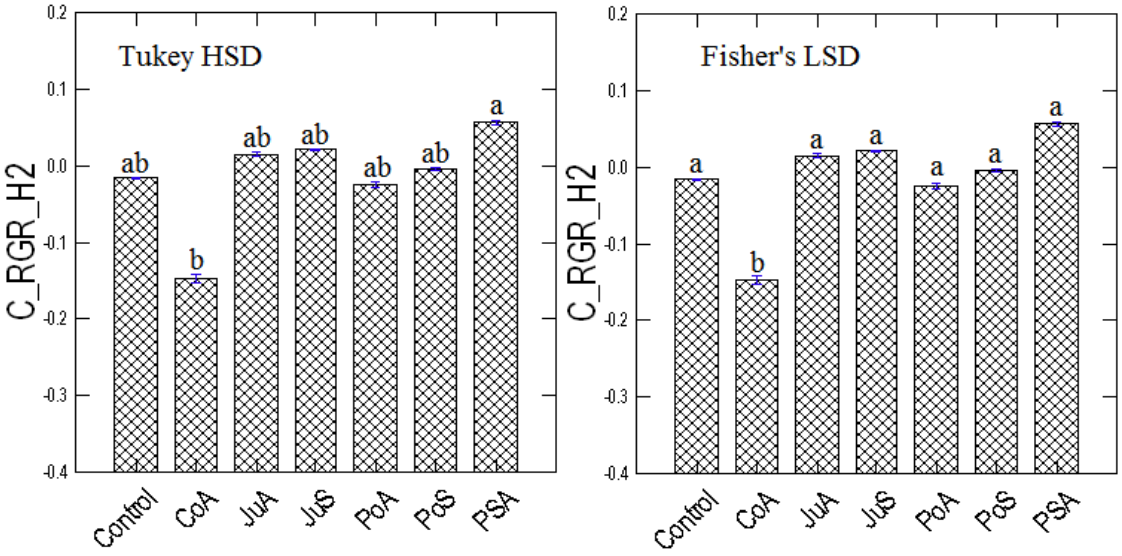

Figure 6. Tukey HSD and Fisher' LSD mean comparison to depict the comparative effect of AMF inoculums and control on RGR-H of *C. africana* seedlings at the 2nd month. Different letters indicate statistically significant pairwise mean difference (P<0.05). The LSD comparison clearly shows that it was only the conspecific inoculum that has statistically significantly lower RGR-H compared to the control.

Table 7. Welch ANOVA for the effect of species and month on RGR-H. "***" indicates there is statistically significant effect (*P*<0.001).

Species	RGR-H			
	Statistic	**df1**	**df2**	**P-Value**
J.procera	0.455	3	54.634	0.715
P.falcatus	46.413	3	49.150	0.000***
C.africana	21.257	3	58.315	0.000***
Month	**Statistic**	**df1**	**df2**	**P-Value**
1st month	2.274	2	46.600	0.114
2nd month	40.200	2	51.164	0.000***
5th month	47.806	2	47.261	0.000***
8th month	11.819	2	51.333	0.000***

the fact that the land is fallowed land where cattle and other domestic animals are allowed to rest urinating and defecating. The high Ave P content in particular could most likely be related to P fertilization legacy effect. P fertilization effect persistently resulted in high Ave P even after many years of fallow with no fertilization (McLauchlan, 2006). Such high Ave P content (151.0 mg/kg) was also reported by Beyene (2003) for a vertic soil of low land Ethiopia.

The AMF spore abundance in the experiment site was significantly lower compared to that of Delelegn et al. (2017) who reported 41-129 spores g^{-1} for different land uses found in a similar kind of ecosystem to that of our experiment site. However, the spore abundance is relatively close to the spore abundance reported on arable land in Ethiopian lowland (5.5 spores g-1) having clay loam soil similar to that of our experiment field site (Belay et al., 2015). It is also comparable to the spore abundance (5.87-13.13 spores g^{-1}) reported beneath coffee shade trees of Bonga, Southwest Ethiopia (Chanie and Assefa, 2013). So, despite the high ave P of the experiment field, spore abundance seems to be comparatively high may be due to 100% soil cover which may have enabled the sporulation of the fungi.

Comparative suitability of J*. procera, P. falcatus*, and *C. africana* for forest restoration in Chancho environs

In this experiment, *J. procera* was found to be

Table 8. Parametric one way ANOVA result for the effect of whole soil AMF inoculation on the RGR-H across the species and months. "**" indicated AMF inoculation has statistically significant effect ($P<0.05$).

Species	Sum of Squares	df	Mean Square	F	P-value
RGR-H of *J. procera* at 1st month	0.089	6	0.015	0.520	0.786
RGR-H of *J. procera* at 2nd month	0.009	6	0.001	0.104	0.995
RGR-H of *J. procera* at 5th month	0.002	6	0.000	0.309	0.925
RGR-H of *J. procera* at 8th month	0.004	6	0.001	0.325	0.916
RGR-H of *P. falcatus* at 1st month	0.937	6	0.156	10.232	0.330
RGR-H of *P. falcatus* at 2nd month	0.170	6	0.028	0.775	0.598
RGR-H of *P. falcatus* at 5th month	0.007	6	0.001	0.473	0.821
RGR-H of *P. falcatus* at 8th month	0.188	6	0.031	1.476	0.234
RGR-H of *C. africana* at 1st month	0.034	6	0.006	0.533	0.777
RGR-H of *C. africana* at 2nd month	0.100	6	0.017	2.645	0.045**
RGR-H of *C. africana* at 5th month	0.120	6	0.020	10.121	0.384
RGR-H of *C. africana* at 8th month	0.143	6	0.024	0.467	0.825

comparatively more suitable than *P. falcatus* and *C. africana* for forest restoration in Chancho environs. A similar result was reported by Abebe et al. (2011) for a similar montane environment. However, unlike their reported survival of *P. falcatus* and *C. africana* for more than two years, in this experiment, both *P. falcatus* and *C. africana* could not even persist for one year with *C. africana* showing death signs shortly after being planted following the severity of cold nights. *J. procera* was the only surviving species at the end of the experiment most likely due to its inherent potential of withstanding frost and drought which is manifested by significantly highest RGR-CD and RGR-H at the 5th month which is the transition from humid condition to drought and frost. The fact that *P. falcatus* has much better RGR-CD and RGR-H at the 2nd month is most likely due to the hand weeding done particularly on *P. falcatus* plots at the end of the first month. At the 1st month tree species mean RGR-CD was statistically significantly variable while RGR-H was not; this indicates maybe all the tree species preferred to extend the root system rather than increasing in height showing common strategy of adapting to the new environment.

Whole soil inoculation effect on seedlings' survival, RGR-CD, and RGR-H at field condition

Whole soil AMF inoculation was found to have no statistically significant effect on seedlings' survival of all the three tree species. It was also found to have no statistically significant effect on both RGR-CD and RGR-H of the three species at all months. However, the CoA inoculum resulted in statistically significant negative effect on RGR-H of C. africana at the 2nd month. Hence, this result is different from other research reports of positive AMF inoculation effect on seedlings' survival and growth (Ouahmane et al., 2006; Manaut et al., 2015). However, it is quite similar to the result obtained from an experiment in California, USA (Aprahamian et al, 2016). In our case, the fact that inoculation did not have effect almost in all the species and months is not to be unexpected considering the high Ave P at the experiment field. Positive AMF inoculation effect is generally known to reduce with the increase in Ave P (Smith et al., 2011). Furthermore, no inoculation effect may have resulted due to sufficient AMF inoculum that may have already existed in the seedlings. According to the experiment result by Pouyu-Rojas and Siqueira (2000), while inoculation of seedlings with AMF during plantation or in the nursery have improved seedlings field survival and growth, re-inoculation of the same AMF for nursery inoculated tree seedlings did not improve seedlings survival and growth any further.

The negative effect AMF inoculum from adult trees has on *C. africana* seedlings RGR-H maybe due to the fact that *C. africana* is an early successional tree species that responds negatively to inoculums from forest source. Rowe et al. (2007) reported that whole soil AMF inoculums from forest origin had positive effect on growth of late successional tree species while it has negative effect on early successional tree species and discuss their result was in agreement with several other reports. However, Kiers et al. (2000) reported that late successional tree species showed statistically significant lower growth and significant higher growth when inoculated with conspecific and non-conspecific AMF inoculums from adult trees respectively. This shows that the negative effect observed here and by Kiers et al. (2000) could mainly be due to the species specific effect of AMF inoculums. Species specific pathogens could also result in negative growth but in their experiment Kiers et al. (2000) have demonstrated that the negative inoculation effect was not due to species specific pathogens but to some another reason which they could not identify.

Conclusion

From this experiment it was clearly seen that *J. procera* is better suited for forest restoration in Chancho environs. Why conspecific inoculum result in negative effect is something to be further investigated. The fact that hand weeding could have resulted in statistically significant higher effect on RGR-CD and RGR-H of *P. falcatus* surpassing *J. procera* is an indication for the importance of tree seedlings aftercare. In this research, although both the AMF status of the tree seedlings used and the AMF composition of the inoculums were attempted to be determined, the necessary data were not generated and hence, were not included in the discussion. Had these data been generated, the basic reasons why for most of the cases inoculation effect was not significant could have been identified. Therefore, future field experiments done on degraded sites with poor soil fertility and with elaborated and detailed measurements of the various variables are required. Future research could also focus on the effect of conspecific inoculums on early successional and late successional tree species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Author would like to thank the AAU plant biology and biodiversity management for providing the necessary funding to carry out this experiment. We would like to appreciate the support of colleagues at the AAU Plant Ecology and Ecophysiology Laboratory and Microbiology Laboratory. The ILRI, Addis Ababa and Dr. Jean Hanson are to be thanked as well for providing us with quality Sorghum x drummodii seeds. The Addis Ababa Administration Environment Protection Authority is also acknowledged for providing us the seedlings used in this experiment.

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