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Suitability of selected legume (*Vachellia* spp.) tree species for forest restoration in the Central Ethiopian highlands

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Abstract. *Asmelash F, Getachew E. 2022. Suitability of selected legume (*Vachellia *spp.) tree species for forest restoration in the Central Ethiopian highlands. Nusantara Bioscience 14: 195-202.* This study aimed to evaluate the comparative suitability of the legume tree species viz., *Vachellia abyssinica* (Hochst. ex. Benth.) Kyal. & Boatwr., *Vachellia etbaica* (Schweinf.) Kyal. & Boatwr., *Vachellia lahai* (Steud. & Hochst. ex Benth.) Kyal. & Boatwr., and *Vachellia seyal* (Delile) P.J.H.Hurter for restoring forests in Central Ethiopian highlands. The suitability of three accessions of *V. seyal* was also compared. The correlation between root nodule number and root Arbuscular Mycorrhizal Fungi (AMF) colonization (RC) and seedlings' growth variables were computed, and the effect of seedling age on nodule number and RC was determined. Seedlings were grown for nine months on degraded local soil in a mesh house in central Ethiopia. We measured shoot height, shoot fresh weight, rooting depth, root nodule number, and RC in the third and ninth months. The one-way ANOVA results indicated that tree species and/or accession (for *V. seyal*) had a significant ($p<0.05$) effect on all the measured variables except shoot height and rooting depth at the ninth month. Generally, nodule number and RC increased with seedling age. However, according to the independent t-test results, significant ($p<0.05$) differences were recorded for *V. abyssinica,* with a 57.16% reduction in nodule number, and *V. seyal* accession-1, with a 418.52% increase in RC. The Spearman's rank correlation results indicated that the correlation between nodule number and RC was weak and non-significant ($p>0.05$) both in the third and ninth months. Based on the measured growth variables, nodule number (N-fixation potential), and RC, *V. etbaica* was the least suitable species for forest restoration in central Ethiopian highlands. The remaining species/accessions are comparably suitable. The *V. abyssinica* lost its comparative fitness with seedling age, maybe because it is a provenance far away from central Ethiopia. However, *V. seyal* accession-3, the furthest provenance, has performed much better. The legume trees of Ethiopia are less studied. Their role as environmental engineers could be better understood by knowing more about their root traits. Therefore, this study could motivate future research in this regard. Long-term experiments are required to consider more legume tree species and provenances in the future.

Keywords: Arbuscular mycorrhizal fungi, nitrogen fixation, nodulation, rhizobium, *Vachellia etbaica*

INTRODUCTION

Forest restoration and conservation are at the core of Ethiopia's climate-resilient green economy strategy; hence it has committed to restoring more than 200 million hectares of forests by 2030 (MEFCC 2018). Most of these forests could be restored on the dry highlands (Pedercini et al. 2021). If this commitment succeeds, it could be crucial to improve the livelihoods of millions of Ethiopians and contribute significantly to climate change mitigation (Strassburg et al. 2020). However, the soils of the Ethiopian dry highlands are too deficient in the essential nutrients (particularly N&P) required for tree/shrub seedlings' field survival, growth, and forest development (Asmelash et al. 2021a). Accordingly, past experiences indicate that despite several forest restoration efforts in these areas, there has been a limited success (Asmelash et al. 2019). One of the reasons for little restoration success could be related to tree species selection (Bekele et al. 2021). Therefore, future forest restoration programs should prioritize tree species selection. In addition, planting legume trees/ shrubs with better N-fixation potential and AMF association could be crucial.

Arbuscular Mycorrhiza (AM) and Rhizobium-Legume (RL) are two of the most important root endosymbiosis that plays key roles in plant nutrition (Barea et al. 2013). Rhizobium can fix N_2 and allow legumes to grow independently of a mineral nitrogen source (Barea et al. 2013; Mahmud et al. 2020). Moreover, legumes could potentially supply N to the associated or succeeding plants (Mahmud et al. 2020). When the soil is deficient in phosphate and nitrates, plants initiate root infection by soil-available AM fungi (Gutjahr 2014). Then, the fungi colonize the root cortex and develop Extraradical Mycelia (ERM), which are very extensive and can significantly increase plants' soil resource acquisition (Asmelash et al. 2016). The role of AM fungi in phosphorus nutrition and forest restoration (Suharno et al. 2017; Asmelash et al. 2016) is well documented. Therefore, legume trees with better N-fixation potential and Arbuscular Mycorrhizal Fungi (AMF) association could preferably be used in forest restoration in the dry Ethiopian highlands.

Currently, about 115 accessions of 20 Vachellia tree and shrub species are conserved in the Ethiopian Biodiversity Institute (EBI) forest gene bank. In the past, there has not been any attempt to evaluate the N-fixation potential and AMF association on these collections.

Therefore, one of the main objectives of this study was to evaluate the N-fixation potential and AMF association of the four *Vachellia* tree species, viz., *Vachellia abyssinica* (Hochst. ex. Benth.) Kyal. & Boatwr., *Vachellia etbaica* (Schweinf.) Kyal. & Boatwr., *Vachellia lahai* (Steud. & Hochst. ex Benth.) Kyal. & Boatwr., and *Vachellia seyal* (Delile) P.J.H.Hurter, conserved in the EBI forest gene bank. Evaluating the comparative N-fixation potential and AMF association of three *V. seyal* accessions was the second main objective of this study. These tree species and accessions were selected since they were the only ones currently conserved in the EBI gene bank suitable for the Ethiopian dry highlands (Hedberg and Edwards 1989).

Previous studies have indicated that the AM and the RL symbioses require a common set of plant genes, constituting a common symbiotic pathway (Barea et al. 2013). Therefore, there could potentially be synergistic AM and RL interactions (Primieri et al. 2021). Hence, we hypothesize that there could be a strong positive correlation between root nodule number and root AMF colonization (RC). Therefore, the third objective of this study was to determine the correlation between nodule number and RC and seedlings' growth variables, i.e., shoot height and shoot fresh weight. Moreover, seedling age could significantly influence root nodulation and/or mycorrhization (Azad et al. 2013; Azad et al. 2016). Hence, determining the effect of seedling age on root nodule number and RC was the fourth objective of the study.

MATERIALS AND METHODS

Seedlings preparation and experiment setup

Seeds of the study species were obtained from the Ethiopian Biodiversity Institute (EBI) forest gene bank (Table 1). The seeds were germinated on filter paper, and then 5 individuals per accession, i.e., 60 seedlings in total, were transplanted on 1-lit plastic pots filled with soil collected from a central Ethiopian highland. The soil is highly degraded (pH = 6.245±0.015, EC = 32.35±0.35ds/m, TN = 0.07%, P (Bray-II) = 4.74 ppm, OM = 5.36%, and CEC = 19.04 m equi/100 g) and its AMF spore abundance was quantified to be 13.95±1.6 g^{-1} (Asmelash et al. 2021a). Treatments were arranged in a split-plot design with third and nine months treatments arranged in separate blocks, and within each block, treatments were arranged in a completely randomized design. Seedlings were watered to field capacity every other day and were grown in the mesh house in EBI, central Ethiopia. The experiment lasted about nine months, counted after seedlings transplantation from 07 October 2021 to 10 June 2022.

Data collection

Data was collected after three and nine months of seedling growth to determine the effect of age on root nodulation and root Arbuscular Mycorrhizal Fungi (AMF) colonization (RC). Seedling shoot height and shoot fresh weight, Relative Growth Rate (RGR) in shoot height and shoot fresh weight, rooting depth, root nodule number, and RC were the measured variables. Shoot height and rooting depth were measured by a ruler, while fresh shoot weight was measured using an analytical balance. The relative growth rate was determined according to Hunt (1990) by the formula RGR= $1/Xt\ (\Delta X/\Delta T)$, where ΔX is the change in seedling growth (Xn-Xt), and between ranked values, Xn is the measurement at the ninth month, Xt is the measurement at the third month, ΔT is the time for the change in days.

The N-fixation potential was estimated by counting the root nodule number (Brockwell et al. 2005). When possible, the different types of nodules were counted separately (Figure 1). Moreover, the pink/red pigmented nodules were counted separately to represent the effective nodules (Azad et al. 2016). Arbuscular mycorrhizal fungi association was estimated by determining root AMF colonization (Asmelash et al. 2021c). AMF colonization was estimated from 100 intersection points observed under a NOVEX light stereomicroscope at (45x) magnification.

Data analysis

Parametric and, when appropriate, non-parametric one-way ANOVA was computed to determine the effect of tree species and accession on the various seedlings' traits measured at the third and ninth months of growth. When a significant (p<0.05) effect was found, pair-wise mean comparisons were carried out between tree species and accessions using Tukey honestly significant difference (HSD) test (p<0.05) or Dunn-Bonferroni test (p<0.05) respectively for parametric and non-parametric ANOVA. An independent t-test (for equal and non-equal variances) was also computed to determine seedling age's effect on nodule number and root AMF colonization (RC). Finally, Spearman's rank correlation was computed to know the correlation between nodule number and RC and the various seedlings' growth variables. R software version 4.1.1 was used to do all the statistical analyses.

Table 1. *Vachellia* species and accessions selected for the study

Species name	Treatm. code	Acc. no.	Altitude (m a.s.l)	Location	Coordinate	Areal distance to the planting site (km)
Vachellia abyssinica (Hochst. ex. Benth.) Kyal. & Boatwr.	VA	244601	1880	Ngele Borena	N5.02° E40.10°	468
Vachellia etbaica (Schweinf.) Kyal. & Boatwr.	VE	20645	1793	North Wollo	N11.73° E39.65°	312
Vachellia lahai (Steud. & Hochst. ex Benth.) Kyal. & Boatwr.	VL	20647	1971	South Wollo	N11.32° E39.68°	270
Vachellia seyal (Delile) P.J.H.Hurter accession-1	VS1	20652	1889	South Wollo	N11.34° E39.68°	272
Vachellia seyal (Delile) P.J.H.Hurter accession-2	VS2	20651	1599	North Shewa	N9.92° E39.85°	149
Vachellia seyal (Delile) P.J.H.Hurter accession-3	VS3	20673	2038	Tigray/Wukro	N13.93° E39.38°	547

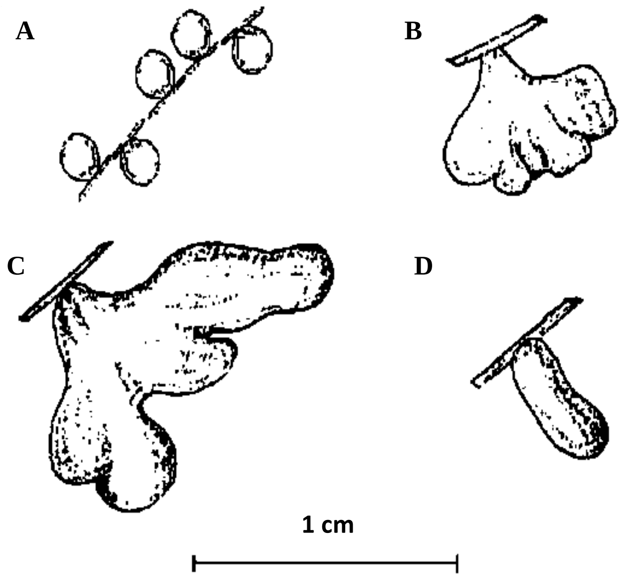

Figure 1. Classification of the shapes of nodules of *Vachellias* (Acacias). A. Globose, B. Coralloid, C. Elongate with branching, and D. Elongate/delicate (Brockwell et al. 2005)

RESULTS AND DISCUSSION

Seedlings growth

None of the seedlings died, and the seedlings' survival was 100% for this experiment. The one-way ANOVA results indicated that, in the third month, tree species and accession had significant effects ($p<0.05$) on both shoot height and shoot fresh weight. The biggest mean shoot height was recorded for *V. abyssinica* (13.5 cm), which was significantly ($p<0.05$) and 91.76% greater than the mean shoot height of *V. etbaica* only. The biggest mean shoot fresh weight was also recorded for *V. abyssinica* (0.51 g), which was significantly ($p<0.05$) and 96.15%, 121.74%, and 131.82%, and 200% greater than the mean shoot fresh weights of *V. seyal* accession-1, *V. lahai, V. seyal* accession-2, and *V. etbaica* respectively. In the ninth month, a significant ($p<0.05$) tree species and accession effect were found for fresh shoot weight, relative growth rate (RGR) in shoot height, and RGR in fresh shoot weight. No significant effect was found for shoot height and rooting depth (Table 2). Although a significant ($p<0.05$) one-way ANOVA result was found for fresh shoot weight, the mean difference between species and accessions was not significant ($p<0.05$) for the Tukey HSD test. However, the biggest mean fresh weight was recorded *V. seyal* accession-3 (0.66 g), and the smallest similar to the third month, was recorded for *V. etbaica* (0.29 g). The biggest mean RGR in shoot height was recorded for *V. seyal* accession-2 (0.0057 cm/cm/day) and was significantly ($p<0.05$) greater than the mean RGR in shoot height of the remaining species and accessions except for *V. lahai*. The RGR in the height of *V. seyal* accession-3 had a decay rate of -0.0001 cm/cm/day. The biggest mean RGR in fresh weight was also recorded for *V. seyal* accession-2 (0.012 g/g/day) and was also significantly ($p<0.05$) greater than the mean RGR in fresh weight of the remaining species and accessions except for *V. lahai*. The RGR in shoot fresh weight of *V. abyssinica* was a decay rate of -0.0003 g/g/day. Regarding the difference in mean growth between *V. seyal* accessions, a significant ($p<0.05$) difference was recorded for mean shoot fresh weight at the third month, RGR in shoot height, and RGR in fresh shoot weight. In comparison, the mean shoot fresh weight of *V. seyal* accession-3 was significantly ($p<0.05$) and 109.10% greater than the mean fresh shoot weight of *V. seyal* accession-2 (third month), it was the mean RGR in shoot height and fresh shoot weight of *V. seyal* accession-2 that was significantly ($p<0.05$) greater than the mean RGR in height and weight of both *V. seyal* accession-1 and 3 (Figure 2).

Nitrogen fixation potential and arbuscular mycorrhizal fungi association

Almost all of the nodules we recorded were globose. No branched elongate nodule was recorded, while few elongate/delicate (10%) and coralloid (1.6%) nodules were recorded. Moreover, all the nodules recorded were not pigmented as viewed externally (Figure 3). According to the one-way ANOVA results, tree species and/or accession was found to have a significant ($p<0.05$) effect on nodule number both at the third and ninth months of seedlings' growth (Table 2). In the third month, no nodule was recorded for *V. etbaica*. However, the biggest nodule number was recorded for *V. seyal* accession-3 (19.2), which was significantly ($p<0.05$) and 200% and 540% more than the nodule number recorded, respectively, for *V. seyal* and *V. lahai* accession-2. In the ninth month also, the highest nodule number was recorded for *V. seyal* accession-3 (33.8), and this was significantly ($p<0.05$) and 5,533.33% greater than the nodule number recorded for *V. etbaica* only. Regarding the mean nodule number difference between the *V. seyal* accessions, a significant ($p<0.05$) difference was recorded in the third month of seedlings' growth but not in the ninth month. Hence, in the third month, the mean nodule number of *V. seyal* accession-3 was significantly ($p<0.05$) and 200% and 242.86% greater than the mean nodule number of *V. seyal* accession-2 and 1 (Figure 2).

Regarding root AMF colonization (RC), the one-way ANOVA results indicated tree species and/or accession effect at the third and nine months of seedlings' growth (Table 2). *Vachellia lahai* had the highest mean RC at the third (39%) and ninth (68%) months. These values were significantly ($p<0.05$) and 1,672.73% greater than the mean RC of *V. etbaica* in the third month and 183.33% and 1,600% greater than the mean RC of *V. seyal* accession-2 and *V. etbaica* at the ninth month. In the third month, the mean RC of *V. abyssinica* was also significantly ($p<0.05$) and 1563.64% greater than the mean RC of *V. etbaica* (Figure 2). Moreover, the mean RC of *V. seyal* accessions was not significantly ($p>0.05$) different both in the third and ninth months (Figure 2). Arbuscules were the predominant form of root AMF colonization, although extra and intracellular spores and hyphae were also observed (Figure 3).

Generally, both nodule number and RC increased by the age of seedlings. However, according to the independent t-test results, except the nodule number of *V. abyssinica* and the RC of *V. seyal* accession-1, none of the remaining tree species and/or accessions had their nodule number or RC significantly ($p<0.05$) affected by seedling age, i.e., three vs. nine months (Table 3). Accordingly, the roots *V. abyssinica* produced significantly ($p<0.05$) and 57.16% fewer nodules in the ninth month compared to the third. On the contrary, the roots of *V. seyal* accession-1 seedlings were significantly ($p<0.05$) and 418.52% more colonized by AMF in the ninth month compared to the third.

Correlation

The correlation between nodule number and root AMF colonization (RC) was a weak positive non-significant ($p>0.05$) both in the third and ninth month (Figure 4). The nodule number, shoot height, and fresh shoot weight correlations in the third month were significant (p<0.05), respectively strong and positive. However, nodule number, shoot height, and fresh shoot weight correlations in the ninth month were weakly positive and non-significant ($p>0.05$). Contrary to this pattern observed regarding nodule number correlation, the RC correlation with shoot height and fresh shoot weight were insignificant ($p>0.05$) and were respectively very weak and weakly positive in the third month. In contrast, in the ninth month, they were significant ($p<0.05$) and were moderate and weakly positive (Figure 4).

Discussion

Using legume trees/shrubs in tropical forest restoration is important to significantly improve restoration success (Gei and Powers 2013; Van Haren et al. 2013; Werden et al. 2018; Mira et al. 2022). In Ethiopia, there have been massive forest restoration programs. However, there has been little attention to tree/shrub species selection, and the deliberate incorporation of the different tree/shrub functional groups, particularly legumes, in forest restoration projects is overlooked. Moreover, very few studies have been conducted to compare the suitability of native legume trees (*Vachellia* spp.) for forest restoration projects. Previously, Tuffer (2017) evaluated the effectiveness of species-specific Rhizobium inoculant on *V. abyssinica*, *V. negrii*, and *V. seyal*. The mean shoot height of *V. seyal* we recorded during the third month of seedlings' growth ranged from 8.48 to 9.86 to 12.4 cm, respectively, for accession-2, accession-1, and accession-3 and was comparable to the mean shoot height (10.6 cm) reported for *V. seyal* seedlings of the same age (Tuffer 2017). However, the mean shoot height of *V. abyssinica* that Tuffer (2017) reported (10 cm) was smaller than the mean shoot height we recorded (13.5 cm).

Table 2. One-way ANOVA result for the effect of tree species and accession on desirable seedling trait

Seedling traits	Third month			Ninth month			
	df	F value	p-value	df	F value	Chi-Sq.	p-value
Shoot height	24	3.9304	0.009585**	24	1.1498	-	0.3621
Fresh shoot weight	24	8.437	0.0001018***	24	2.7242	-	0.04363*
Rooting Depth	-	-	-	24	1.7401	-	0.1638
RGR in shoot Height	-	-	-	24	14.014	-	0.000001844***
RGR in fresh shoot weight	-	-	-	24	20.796	-	0.00000005158***
Nodule number	24	7.1402	0.0003224***	24	-	20.483	0.001014**
Root AMF colonization	24	3.9822	0.009009**	24	4.288324	-	0.00628**

Note: Significant species and/or accession effect at *0.05, **0.01, and ***0.001. RGR: relative growth rate

Table 3. Mean nodule number, AMF root colonization, and the effect of seedling age was tested by independent t-test

Tree species/accession	Nodule number (Mean±std.Err.)		Independent t-test p-value	Root AMF colonization (Mean±std.Err.)		Independent t-test p-value
	3rd month	9th month		3rd month	9th month	
Vachellia seyal (Delile) P.J.H.Hurter -accession 1	5.6±2.04	12.6±5.22	0.247	5.4±3.71	28±8.89	0.04703*
Vachellia etbaica (Schweinf.) Kyal. & Boatwr.	0±0.00	0.6±0.24	0.9648	2.2±1.56	4±1.70	0.4584
Vachellia lahai (Steud. & Hochst. ex Benth.) Kyal. & Boatwr.	3±1.14	3.4±0.60	0.7641	39±11	68±11.47	0.1055
Vachellia seyal (Delile) P.J.H.Hurter - accession 2	6.4±2.73	13.4±1.94	0.07004	18.4±6.27	24±11.73	0.6849
Vachellia abyssinica (Hochst. ex. Benth.) Kyal. & Boatwr.	16.8±3.48	7.2±1.24	0.03184*	36.6±11.87	29.6±10.57	0.6714
Vachellia seyal (Delile) P.J.H.Hurter - accession 3	19.2±5.01	33.8±10.54	0.2462	16.8±6.14	34±11.97	0.237

Note: *significant diffrence

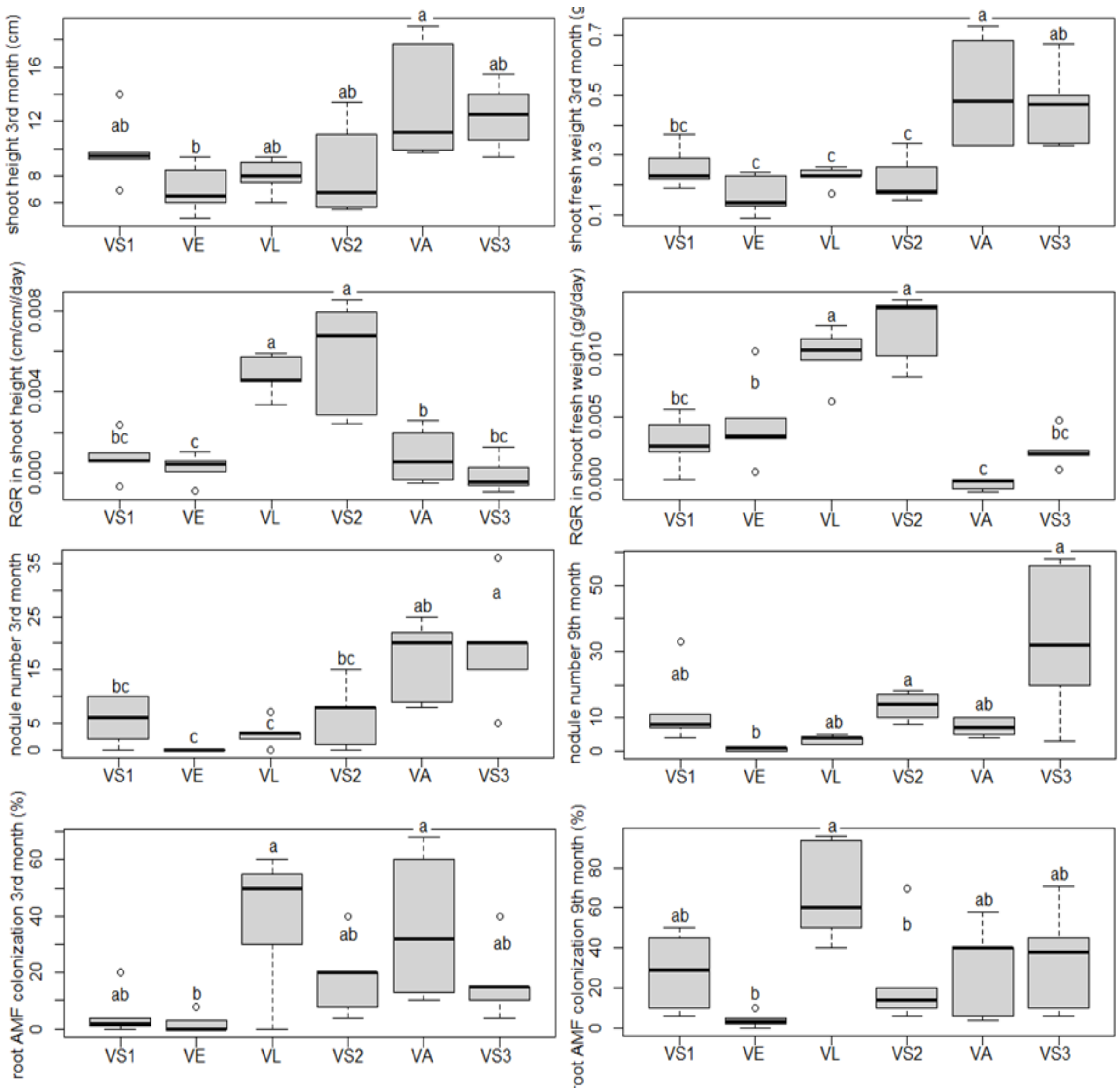

Figure 2. Mean difference in seedling traits between tree species and accessions. Data containing means with a significant difference for Tukey HSD (p<0.05) or the Dunn-Bonferroni test (p<0.05) do not contain similar letter labels. AMF= Arbuscular Mycorrhizal Fungi, VS1= *Vachellia seyal* accession-1, VE= *Vachellia etbaica*, VL= *Vachellia lahai*, VS2= *Vachellia seyal* accession-2, VA= *Vachellia abyssinica*, VS3= *Vachellia seyal* accession-3, RGR=Relative Growth Rate

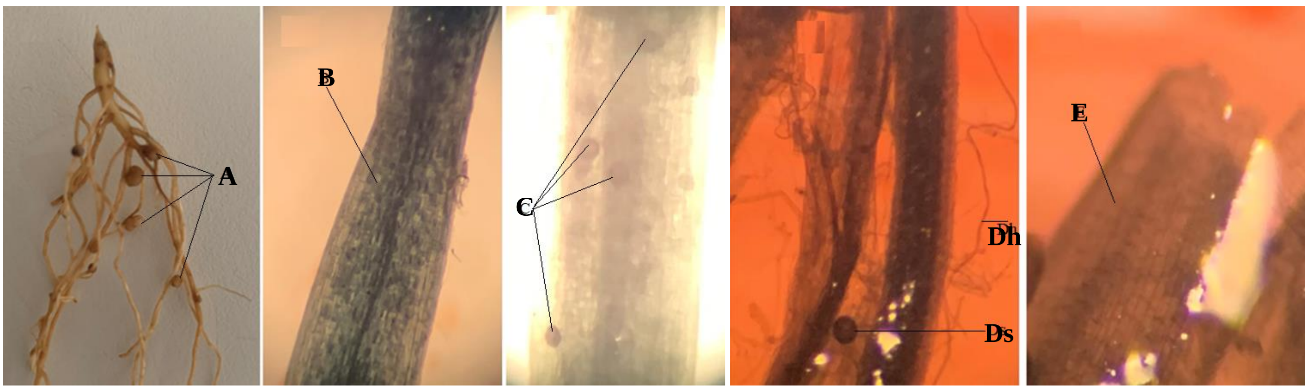

Figure 3. Picture to show nodulation and arbuscular mycorrhizal fungi colonization. A: Nodulation of the root (globose, big sized), B: AMF colonization with arbuscules, C: AMF colonization with intraradical spore, Ds: AMF colonization with extraradical spore, Dh: AMF colonization with extraradical hyphae, E: No AMF colonization. Pictures are not on the scale

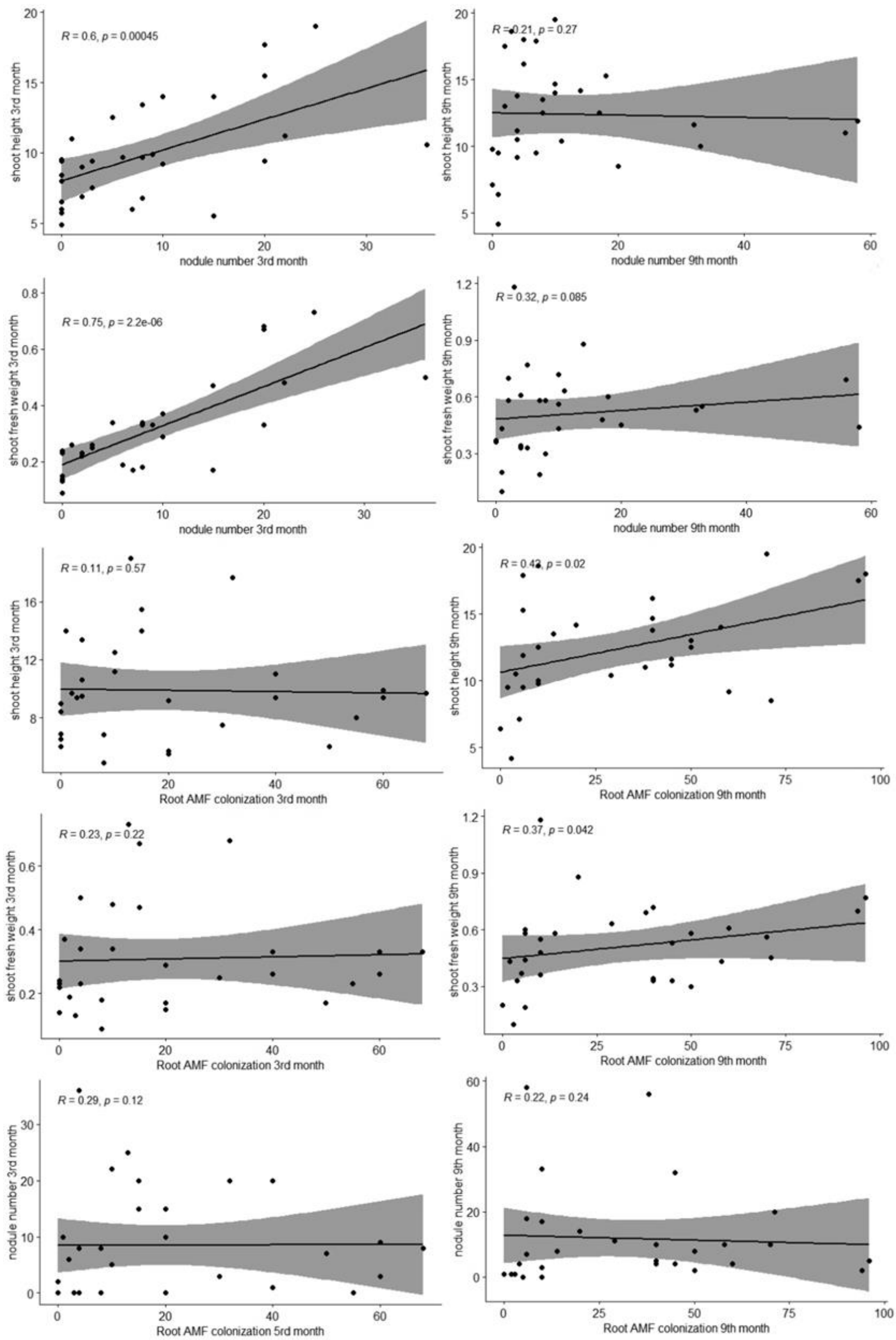

Figure 4. Spearman's rank correlation between root nodule number, root arbuscular mycorrhizal fungi colonization, seedling shoot height, and shoot fresh weight

We used nodule number and pigmentation to determine N-fixation potential. Our pigmentation assessment was ineffective as we only observed nodule color from the outside. Effective nodules are those pigmented red/pink on the inside and not outside (Brockwell et al. 2005) and could be observed by cutting the nodules (Hultman 2018) or using light microscopy (Ardley et al. 2013). However, nodule number is still an important variable in determining N-fixation potential. Accordingly, an increase in mean nodule number has been reported to increase the mean N-fixation potential (computed as the shoot dry mass ratio between inoculated and N^+ fertilized plants) of three months old *V. abyssinica* and *V. seyal* seedlings (Tuffer 2017). Nodule number and nodule position in the root are tightly controlled by the plant (Ferguson et al. 2010), and thus, nodule number could be an important variable in comparing legume trees. The mean nodule number we recorded for *V. seyal* (5.6 to 6.4 to 19.2 for the three accessions) and *V. abyssinica* (16.8) in the third month are very much smaller than the mean values, i.e., 42 and 37, reported by Tuffer (2017) respectively for the seedlings of the same species of similar age. These differences could be attributed to seedlings being inoculated with species-specific rhizobium bacteria in the previous experiment. Elongate/delicate nodules, i.e., cylinder-shaped nodules, are considered to be more involved in N-fixation in Vachellias (Acacias), while globose (spherical) nodules, particularly the small sized, are ineffective (Brockwell et al. 2005). Therefore, the fact that we recorded a few elongated nodules could indicate that our study species had low N-fixation potential in our experiment condition, resembling the central Ethiopian highlands.

We recorded a general trend in an increase of nodule number in the ninth month compared to the third. Similarly, a general increase in nodule number has been reported for seedlings of other legume tree species (Azad et al. 2013; Azad et al. 2016). On the contrary, in the case of *V. abyssinica*, we recorded a significant (p<0.05) reduction in nodule number with an increase in seedling age. That also coincided with a decay rate in fresh weight (-0.0003 g/g/day) recorded for *V. abyssinica*. Nodulation/N-fixation could be affected by several factors that also affect seedlings' growth. For example, leaf removal has significantly reduced nodule growth and N-fixation (Marschner 2012). Similarly, other factors such as light, soil nutrients, soil moisture, and temperature could also significantly reduce nodule number and N-fixation (Brockwell et al. 2005). Hence, in our case, weather change-induced reduction in light intensity and soil temperature and hence, leaf shedding (observed during watering) could have resulted in the nodule number reduction, particularly by the *V. abyssinca* seedlings. That could also be related to the particular provenance (accession no: 244601) being located at the hottest range of the species, which is also far from central Ethiopia.

In this experiment, we also evaluated root AMF colonization (RC) to determine the legume tree species or accession with a better potential for N-fixation and phosphorus acquisition. The mean RC levels we recorded in most cases vary from the mean RC levels reported previously. Accordingly, the mean RC we recorded for *V. etbaica* (2.2% and 4%, respectably for the third and ninth months) is very small as compared to the mean RC reported (73%) for adult individuals of the same species in north Ethiopia (Birhane et al. 2017). Whereas the mean RC we recorded for *V. abyssinica* at the third (36.6%) and ninth (29.6%) months were much higher than the mean value previously reported (18.3%) by (Zerihun et al. 2013) for adult *V. abyssinica* trees in central Ethiopia, these mean RC values were very much lower than the mean RC reported (96%) for *V. abyssinica* seedlings (Asmelash et al. 2021c). Variable mean RC levels have also been reported previously for adult *V. seyal* trees, 78.2% (Birhane et al. 2017) and 42.1%, 48.8%, and 96.4% (Zerihun et al. 2013). Likewise, we recorded variable mean RC at the third and ninth months of seedling growth. Regarding *V. lahai*, (Birhane et al. 2017) previously reported a mean RC of 83% for adult trees which is much higher than the mean RC we recorded in the third month (39%) but relatively comparable to the mean RC we recorded at month nine (68%). Root AMF colonization is determined more by the host species (John St. 1980; Schüßler et al. 2016; Silva-Flores et al. 2019). Therefore, the variable and wide range of RC we recorded and previously reported may indicate that the study species have a wide range of RC. Moreover, soil property could also be responsible for these variable RC values. Root AMF colonization is generally lower for degraded soils than fertile or virgin soils (Asmelash et al. 2021b). Hence, the generally low mean RC we recorded for the study species could be expected since the potting soil used in this experiment was highly degraded. Except for the non-significant (p>0.05) reduction of RC by *V. abyssinica*, the remaining RC values recorded increased with age and particularly significantly (p<0.05) for *V. seyal* accession-1. Root AMF colonization and age relationships could vary widely between host species (Asmelash et al. 2021c). However, in our case, there seem to be more or less similar RC and age relationships for the study species and accessions. That could be because the species are taxonomically related, being from a similar genus (John St. 1980).

In the third month, nodule number correlated significantly (p<0.05) with shoot height and fresh shoot weight, while RC did not. On the contrary, in the ninth month, RC correlated significantly (p<0.05) with shoot height and shoot fresh weight, not nodule number. That may indicate that with an increase in seedlings' age, rhizobia's role in the legumes' nutrition declines while the role of AMF increases. Moreover, nodule number and root AMF colonization were not significantly (p>0.05) correlated in the third and ninth months. That could be because the nodule numbers we recorded were not all active/pigmented inside or because nodule number/RC correlations are species-dependent.

In this study, we evaluated the variation in N-fixation potential (nodule number) and arbuscular mycorrhizal fungi (AMF) association determined by root AMF colonization (RC) between *V. abyssinica*, *V. etbaica*, *V. lahai*, and *V. seyal* (represented by three accessions). We also evaluated the comparative growth of these species and accessions on the degraded soil of central Ethiopia in a mesh house in central Ethiopia. We also determined the correlation between nodule number and RC and between nodule number and RC with seedlings' shoot height and fresh weight. We also determined the effect of seedling age on nodule number and RC. Based on growth, nodule number, and RC, *V. etbaica* (accession number: 20645), could be considered the less suitable species for forest restoration in central Ethiopian highlands. The remaining species and accessions have distinct qualities considering growth, N-fixation potential, or AMF association. *V. lahai* is the most suitable if considering mainly AMF association.

V. seyal accession-3 is the furthest provenance to central Ethiopia; it performed better in N-fixation potential, growth, and AMF association than provenances collected from near central Ethiopia. Hence, the general assumption of using local provenances in forest restoration should be evaluated per tree species. The legume trees of Ethiopia are less studied; particularly, their role as environmental engineers could be better understood by knowing more about their root traits. Therefore, this study could motivate future research in this regard. Long-term experiments are important as experiments on seedlings alone, although crucial, may not be sufficient. In this experiment, a few legume tree species and provenances were evaluated. The comparative suitability of more tree species and provenances/ accessions is required in the future.

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