Original Research Article

Exploration and assessment on the agronomic requirement of *Tavernieraabyssinica*A. Rich: a critically endangered medicinal plant of Ethiopia

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ABSTRACT

We determined the distribution and abundance of *Tavernieraabyssinica*A. Rich, a critically endangered endemic medicinal plant of Ethiopia, in the Shewa floristic region, Ethiopia. We also carried out a mesh-house experiment to know whether *T. abyssinica* is able to survive and grow in any soil. From the nine potential locations we made exploration, *T. abyssinca*ppulations were found only in the two, Lemen and Mojo. The abundance of mature individual was estimated to be more than 600/hectare. The one-way ANOVA and mean comparison (Tukey or Dunn-Bonferroni) results indicated that seedlings grown on soil collected from Lemen and Mojo were significantly (p<0.05) taller and heavier than those seedlings grown on Addis Ababa soil (where the species was never reported to grow). We report for the first time that *T. abyssinica* is N-fixing and arbuscular mycorrhizal, at least at the seedling stage. Seedling grown on the Addis Ababa soil were not colonized by arbuscular mycorrhizal fungi while those grown on Lemen and Mojo soils were. Although the estimated abundance of mature individuals was high, there is continued exploitation of the species and habitat loss is imminent. Therefore integrated conservation program by way of ex situ conservation, in situ conservation, and cultivation should be implemented. *Tavernieraabyssinica* could be cultivated in areas with leptosol and degraded vertisol soils with slightly acidic to basic pH. Arbuscular mycorrhizal fungi could have a significant role in the conservation and cultivation of the species.

Keywords: Analgesic property, antipyretic property, medicinal plants (MPs), leptosol, *Taverieraabyssinica*, vertisol

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1. INTRODUCTION

From the recorded 6000 vascular plant species of Ethiopia, the 887 are medicinal plants (MPs) of which, the 2.7% are endemicto Ethiopia [1]. *Tavernieraabyssinica* A. Rich is one the most valuableendemic MPs of Ethiopia [2,3]. Owing to its traditional use to treating sudden illnesses of all sorts, *T. abyssnica* is locally named “Dingetegna” to mean “sudden remedy”. The dried slender root (or sometimes stem) is chewed and the juice swallowed or the smoke of these plant parts is inhaled to treat, among others, fever, stomach ache, Colic, and sudden illness due to evil spirits [3]. Analgesic and antipyretic properties of the *T. abyssinica* root extract has been proved to be significant [4]. Its potential effect against stomachache has been demonstrated [5]. It also possesses strong nematicidal and weaker cytotoxic and antimicrobial effects [6]. The root extract was found to contain isoflavonoids [7] which may have potential anticancer effects [8].

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Unsustainable harvest and habitat loss due to agricultural expansion have resulted in the significant decline of the *T. abyssinica* natural populations. Hence, *T. abyssinica* is considered to be one of the critically endangered endemic plants of Ethiopia [9]. In light of its endemism, conservation status, and current and future economic potential, *T. abyssinica* conservation should therefore beamong the primary biodiversity conservation priorities in Ethiopia.However, there has been little effort of conserving the species. The current distribution and abundance of *T. abyssinica* populations is also not well known.

One of the viable mechanisms of conserving endangered or over-exploited MPs is to cultivate them [10, 11].Likewise, *T. abyssinica* is one of MPs recommended for cultivation [2].In the past, there have few research works relevant to the conservation and cultivation of *T. abyssinica*. The seeds of *T. abyssinica* were determined to be orthodox and mechanical or chemical (98% sulfuric acid) treatments were known to be effective methods to break seed dormancy [12]. Effective in-virto propagation protocols of the species have also been developed [13, 14]. However, the agronomic requirement of the species is not known. Therefore, the objectives of this study

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wereto: 1) explore for the natural populations of *T. abyssinica*in the Shewa floristic regionand 2) to carryout preliminary assessment on the agronomic requirement of the species.

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2. MATERIAL AND METHOD

2.1. Description of *Tavernieraabyssinica*

Tavernieraabyssinica A. Rich (Fabaceae/Papilionoideae) is a single foliolate or very rarely, pinnately 3-foliolate shrub or shrublet reaching up to 2 m in height. Leaflets are glabrous above while appressed-pubescent beneath. Racemes 2-8-flowered; rhachis and peduncle together c 3-25 mm long. Calyx apressed-pubescent outside; lobes equaling or longer than tube. Corolla 12-17 mm long, dark pink to purplish red. Pods are stipitate (stalk bearing), 1-3 segmented, finely pubescent and spiny (c 1.5 mm long). Young stems are covered with densely appressed-fine hairs [15, Fig. 1].

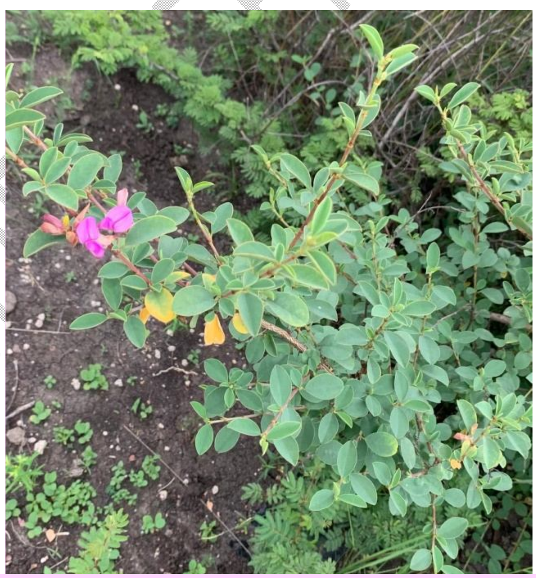

Figure 1: *Tavernieraabyssinica* A. Rich. (Photo by FissehaAsmelash). The flower of *T. abyssinica* is Papilionaceous with diadelpous stamen.

2.2. Exploration of *Tavernieraabyssinica*natural populations

Based on the Flora of Ethiopia and Eritrea[15], herbarium records, and interview with *Dingetegna* vendors in Merkato (the biggest market in Addis Ababa and Ethiopia), we determined that *T. abyssinica* populations were found in Shewa and Tigray floristic regions of Ethiopia. Hence, to assess the current conservation status of *T. abyssinica*, we made explorationsin the Shewa floristic region; particularly in the localities of Adadi Mariam, Aliyu Amba, Butajira, Debralibanos, Deneba, Ensaro, Gohatsion, Lemen, and Mojo.

At each locality, relevant government offices and experts were contacted and together, local key informants (farmers and traditional healers) were identified for interview. The species was described to the key informants by also telling them the local name and showing an illustration of the species based on [15]. When key informants say they recognize the species, they were asked to locate *T. abyssinica* populations. For those places where the key informants where able to locate *T. abyssinica* populations, the name of the locality, geographic coordinate, altitude, and *T. abyssinica*abundance were recorded. Abundance was determined by counting *T. abyssinica* individuals in 30 by 30 plots laid at each site where a *T. abyssinica* population was located.

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2.3. Assessment on the agronomic requirement of *Tavernieraabyssinica*

2.3.1. Mesh-house experiment design

To know if *T. abyssinica* can survive and grow on soils other than where its natural populations are found, a mesh-house experiment was carried out in the Ethiopian Biodiversity Institute, Shewa floristic region, Addis Ababa. Based on the *T. abyssinica* exploration result (Table 2), Lemen and Mojo were identified to be the locations where *T. abyssinica*natural populations are found. Hence, soil from Lemen and Mojo was collected for the mesh-house experiment. Another soil from Addis Ababa where no *T. abyssinica* population has ever been reported was also collected. After four months of seedlings growth, survival rate, growth, and root traits (nodulation and mycorrhization) of *T. abyssinica*were compared. Hence, a preliminary assessment on the agronomic requirement of the species was made.

Seeds collected from the Mojo population were germinated on filter paper according to [12]. Germinated seedlings were then transplanted on eighteen 1-liter plastic pots filled with the Addis Ababa, Lemen, and Mojo soil (six pots each). On each of the pot, two *T. abyssinica* seedlings were transplanted making the total number of seedlings in the experiment 36. Hence, in this experiment,the treatment was soil (Table 1) and the three soil types were arranged in a completely random design. The experiment lasted for four months from March 31, 2022 to July 31, 2022.

Table 1: Soil type and physiochemical property of the potting soil used (Based on World Soil Information Service data base available online: https://soilgrids.org/)

Soil characteristics	Potting soil type		
	Addis Ababa	Lemen	Mojo
Soil type	Luvisol	Vertisol	Vertisol
Bulk density (cg/cm3)	128	128	132
Sand content (g/kg)	121	208	219
Silt content (g/kg)	489	307	401
Clay content (g/kg)	376	490	385
pH	6.2	6.7	7.1
Organic carbon (dg/kg)	299	188	174
Total nitrogen(cg/kg)	209	164	162
CEC (mmol(c)/kg	320	397	513

Note: soil property is for the5-15cmof soil depth

2.3.2. Growth and root traits measurement

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After four months of growth, seedlings survival rate and growth, viz., leaf number, shoot height, shoot fresh weight, and root fresh weight were determined. Survival was computed per pot as (number of living individuals/ total number transplanted)*100. Leaf number was determined by counting leaves per plant. Shoot height was measured by a ruler while shoot fresh weight and root fresh weight were measured by analytical balance. Since, part of the root was delicate, some fine root have remained attached to the soil. Hence, the root fresh weight measured was mainly on the root part that was effectively pulled out from the soil matrix and which comprises the major proportion of the root. Two of the most important root traits (nodulation and mycorrhizal association) were also determined by counting the number of root nodules and by measuring root arbuscular mycorrhizal fungi (AMF) colonization. Since nodule size is an important predictor of N-fixation potential [16], we counted the nodules that were easily visible with the necked eyes. Very small nodules that were not developed well were not counted. Root AMF colonization was determined by the gridline intersection method on 100 intersection points [17]by using a NOVEX light stereomicroscope (45x). Roots were first cleared in 10% KOH[18] and stained and de-stained by the ink and vinegar technique [19] using black Hero ink as stain [20].

2.3. Data analysis

One-way ANOVA was computed to know the soil preference of *Tavernieraabyssinica*. The data were first checked for the equality of variances and parametric ANOVA or Kruskal–Wallis test were computed to know the effect of soil. When significant effect (p<0.05) was found, means were computed using Tukey honestly significantdifference (HSD) orDunn–Bonferroni tests (p<0.05) respectively for the parametric ANOVA orthe Kruskal–Wallis test. All the statistical analysis was carried out using the R software version 4.1.1.

3. RESULTS

3.1. The conservation status of *Taverieraabyssinica*

From the ninelocations where exploration was carried out, *Tavernieraabyssinica* populations were found only in Lemen and Mojo (Table 2). In Mojo, three populations were located and *T. abyssinica* grows abundantly (more than 500/hectare). In Lemenalso, three populations were located. However, the *T. abyssinica*abundance was much lower than Mojo (more than 70/hectare). The *T. abyssinica* populations were found in highly degraded sites with heavy clay soil. In Lemen, the species is mostly found growing solitary. However, in Mojo area, it was found growing in thickets with different plantspecies (Fig. 2).

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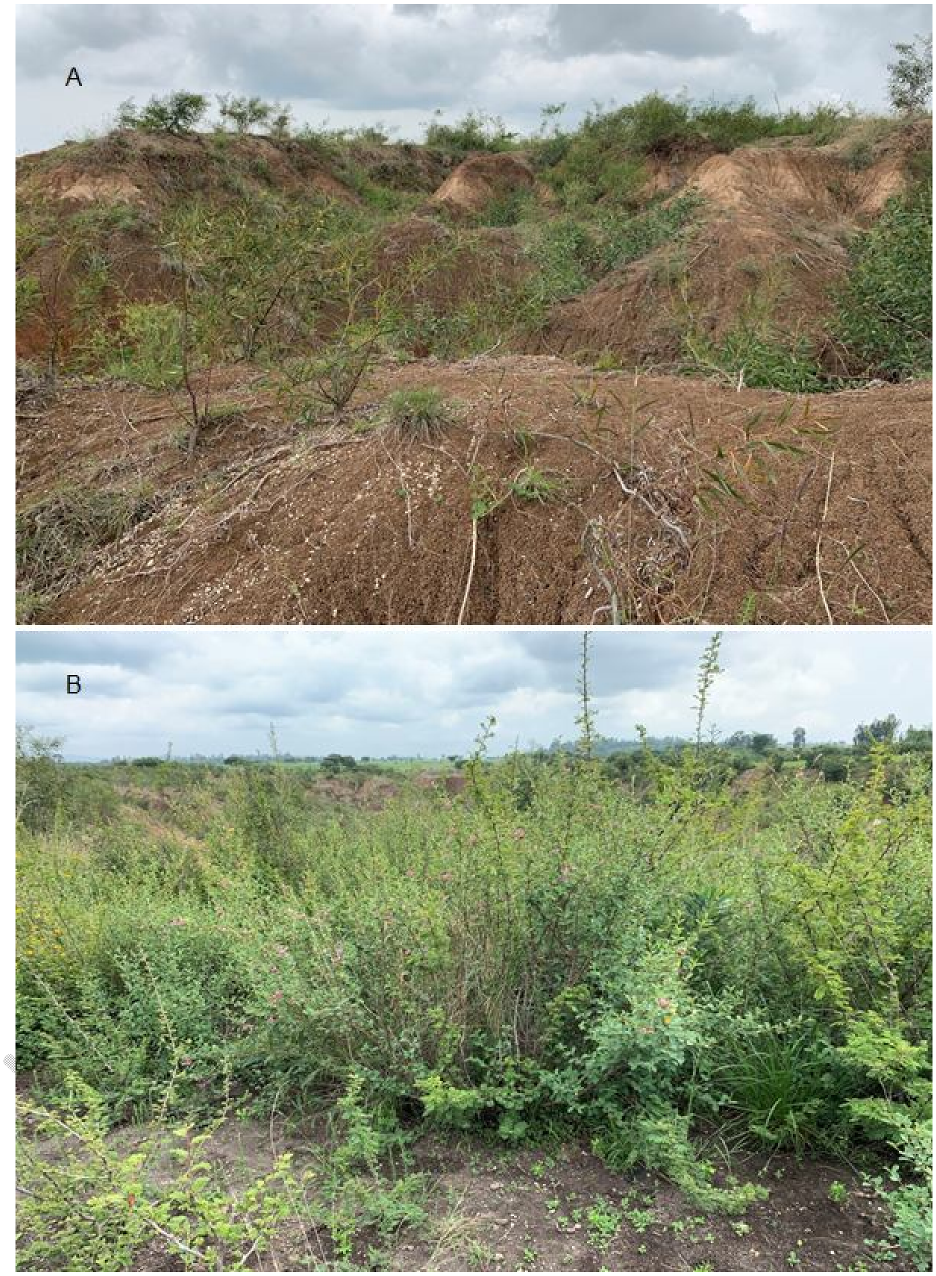

Figure 2:*Taverneriaabyssinica* grows in highlydegraded sites; *Acacaiasaligna*planted to reclaim the site is visible(A) and it can grow together with other plantspecies forming a thicket. Those plants with flowers (B) are *T. abyssinica* individuals.

Table 2: The location and abundance of *Taverniearabyssinica* populations in parts of the Shewa floristic region

Location	Region	Zone	*Tavernieraabyssinica* presence and estimated abundance	
			Presence	Abundance
Adadi Mariam	Oromia	South West Shewa	No	Nil[§]
Aliyu Amba	Amhara	North Shewa	No	Nil
Butajira	South	Butajira	No	Nil
Debralibanos	Oromia	North Shewa	No	Nil
Deneba	Amhara	North Shewa	No	Nil
Ensaro	Amhara	North Shewa	No	Nil
Gohatsion	Oromia	North Shewa	No	Nil
Lemen	Oromia	South West Shewa	Yes	78/hectare
Mojo	Oromia	East Shewa	Yes	556/hectare

[§]local informants described the species correctly and insisted the species is present; however they were not able to locate a single site where *Tavernieraabyssinica* is growing.

3.2. The agronomic requirement of *Tavernieraabyssinica*

Generally, seedlings grew better on the Mojo and Lemen soil and in a descending order while they grew poorly on the Addis Ababa soil (Fig. 3). After four months of growth, the survival rate of *T. abyssinica*seedlings was 100%, 92%, and 83%, respectively for Mojo, Lemen, and Addis Ababa soils. According to the one-way ANOVA result, these survival rates were not significantly (p>0.05) different (Table 3). However, significant (p<0.05) effect of soil was found for all the remaining seedling variables measured, viz., leaf number, shoot height, shoot fresh weight, root fresh weight, root nodule number, and root AMF colonization (Table 3). The seedlings grown on the Mojo soil produced the highest leaf number which was significantly (p<0.05) and 23.48% and 305.71% more than the mean leaf number of the seedlings grown on the Lemen and Addis Ababa soils respectively. Similarly, the highest shoot height, shoot fresh weight, and root fresh weight was also recorded for seedlings grown on the Mojo soil to be followed by the seedlings grown on the Lemen and Addis Ababa soils respectively. Whereas the highest nodule number was recorded for seedlings grown on the Lemen soil, it was for the seedlings grown on Mojo soil that the highest root AMF colonization (RC) was recorded. The nodule number of seedlings on the Lemen soil was significantly (p<0.05) and 236.64% and 1233.33% greater than the mean nodule number of seedlings grown on Mojo and Addis Ababa soils respectively. The RCof seedlings on the Mojo soil was not significantly (p>0.05) greater than the mean RC of the seedlings grown on the Lemen soil. The RC of seedlings gown on both Mojo and Lemen soil were however, significantly (p<0.05) greater than the mean RC of seedlings grown on the Addis Ababa soil. The roots of the seedlings grown on the Addis Ababa soil were not colonized by AMF (Table 3). Root nodules recorded were mostly oval but there were also elongate/cylindrical and spherical/globose nodules. In the case of RC Vesicles were the frequently observed structures (Fig. 4).

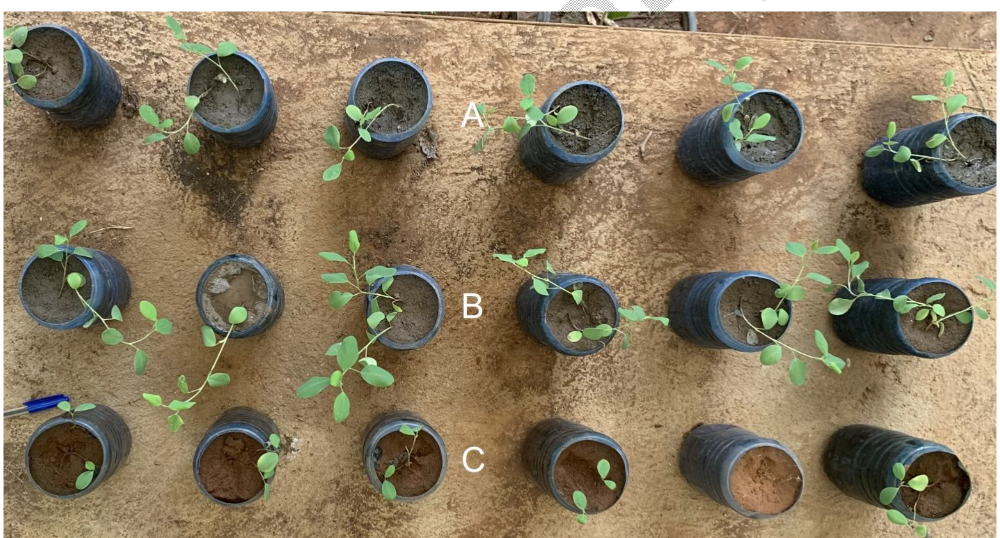

Figure 3: *Tavernieraabyssinica* seedlings after four monthsof growth in a mesh-house in the Ethiopian Biodiversity Institute. Row A=Lemen soil, Row B=Mojo soil, and Row C=Addis Ababa soil. Seedlings seem to grow poorly on the Addis Ababa soil.

Table 3: One-way ANOVA results and mean comparison

Variables	Mean (±SE) values on the different soil			ANOVA	
	Addis Ababa	Lemen	Mojo	F	Chi.sq.
Survival rate (%)	83.0(±17) [ns]	100.0(±0.0) [ns]	92.0(±8) [ns]	0.6	-
Leaf number	1.75(±0.25) [c]	5.75(±0.39)	7.1(±0.82) [a]	-	24.312***

		^{b}			
Shoot height (cm)	5.77(±0.31) ^{c}	9.44(±0.5) ^{b}	12.25(±0.9) ^{a}	25.421***	-
Shoot fresh weight (g)	0.15(±0.01) ^{c}	0.43(±0.04) ^{b}	0.65(±0.06) ^{a}	32.617***	-
Root fresh weight (g)	0.11(±0.01) ^{b}	0.18(±0.02) ^{a}	0.24(±0.04) ^{a}	5.5685*	-
Root nodule number	0.6(±0.6) ^{b}	8.0(±1.1) ^{a}	2.2(±1.0) ^{b}	17.796***	-
Root AMF colonization (%)	0.0((±0.0) ^{b}	26.17(±7.1) ^{a}	45.0(±13.3) ^{a}	-	10.753**

*significant at $p<0.05$, ** significant at $p<0.01$, and *** significant at $p<0.001$. Means labeled with different lettersacross columns are significantly different for TukeyHSD or Dunn-Bonferronitestes ($p<0.05$), “ns” indicates means are not significantly different ($p>0.05$).

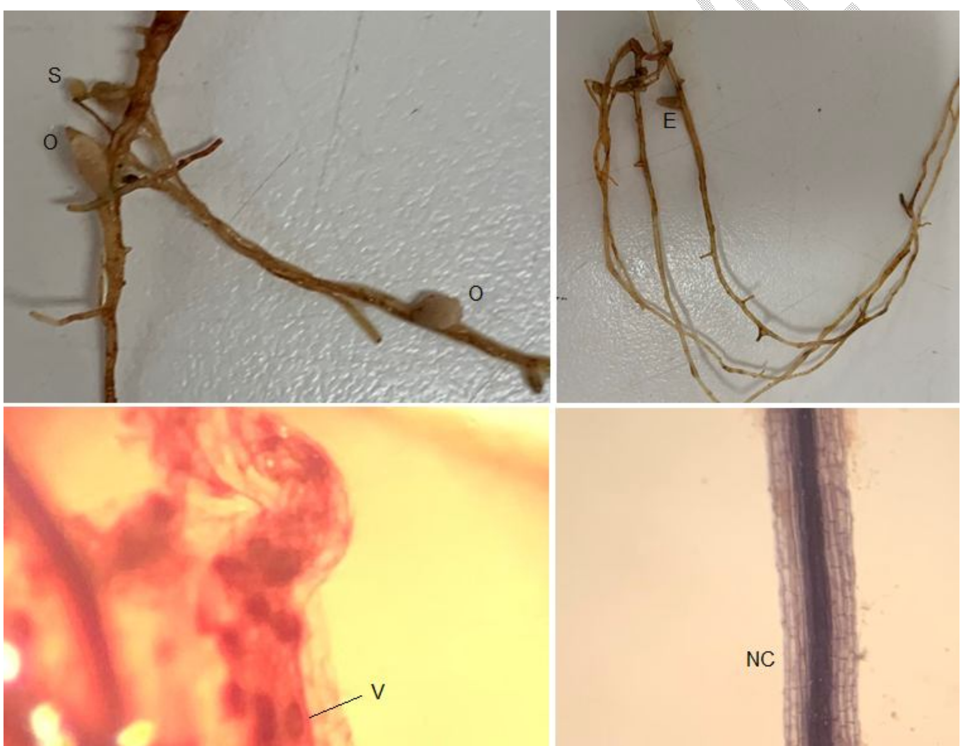

Figure 4: Nodulated rootsof *Tavernieraabyssinica* seedlings above and arbuscular mycorrhizal fungi (AMF) colonization below. E=elongate nodule, O=oval nodule, S=spherical nodule, V= vesicles (root colonized with AMF), NC=root not colonized with AMF. Photos are not with scale.

4. DISCUSSION

Tavernieraabyssinica was categorized to be an IUCN "critically endangered" species not based on abundance of mature individuals but by its distribution, experts' judgment of population decline, and actual or potential levels of exploitation [9]. Here, we have determined an estimated abundance of *T. abyssinica* mature individuals (250-1000) which if used alone, will put the species under the "vulnerable" category. Since we found *T. abyssinica* populations in two locations (six sites), one of the criteria, i.e., existing only at a single location, that was used previously to designate the species as critically endangered [9] is also not valid currently. This could be more so if the number of mature individuals were to be accounted throughout Ethiopia (Table 4). However, we observed the species is still under exploitation for its root and in Adadi Mariam, we noticed its habitat has been changed to cultivated land.The Lemen populations were also found interspersed within agricultural lands, which indicate agricultural expansion in to the specie's habitat have taken place. Moreover, we have observed frequent uprooting of the species particularly in the Lemen area. Hence, although the number of mature individuals could potentially be significantly high, considering the current and potential future exploitation and considering the high risk of habitat loss, the current critically endangered designation could still be appropriate. In Mojo area, we observed some of the *T. abyssinica*populations growing in thickets. This corroborates the flora record that described the species as a bush land species [15]. Therefore, *T. abyssinica* could be conserved in situ in protected bushlands/forests.

Available data indicate that *T. abyssinica* grows on leptosols and vetrisols (Table 4). Our results corroborate this data. Hence, in the mesh-house condition in the Ethiopian Biodiversity Institute (2400 m altitude), *T. abyssinica*although survived well, it did not grow fit on the

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Luvisolcompared to the vertisol. Hence, soil type could be an important agronomic requirement of *T.abyssinica.*Leptosols and vertisols could have better CEC and copper content compared to Luvisols [21]. Hence, soil type in general and CEC and essential nutrients such as copper in particular, could be important factors to determine *T. abyssinica* growth. The soil pH could be another important factor. Seedlings grew better on the Mojo soil with a slightly basic pH compared to the Lemen and Addis Ababa soils with slightly acidic and acidic pH values. This may indicate that the species prefers slightly basic soils than acidic ones. The comparative better growth of *T. abyssinica* seedlings on the Mojo soil could also be due to home soil advantage. This is because, the seeds used in this study were collected from the Mojo populations.

Table 4: Soil type and pH of sites where herbarium record *Taverneriaabyssinica* was found (Based on https://soilgrids.org/).

	Location	Floristic region	Soil type	pH
8	Mekelle city	Tigray	Leptosol	7.9
6	Adigudem-B	Tigray	Vertisol	7.8
1	Adigudem-A	Tigray	Vertisol	7.7
3	Hagereselam	Tigray	Leptosol	7.7
7	Adi Amedy	Tigray	Leptosol	7.7
5	Mekelle/Giba plane	Tigray	Leptosol	7.5
2	Gijet	Tigray	Leptosol	7.3
4	Abala	Tigray	Leptosol	7.2
11	Gebraguracha	Shewa	Leptosol	6.4

Note: exploration to all these locations was not possible due to security reasons

Leptosiols are problematic soils for crops growth [22]. *Tavernieraabyssinica* thrives well on such soils and degraded verisols. Hence, the species must have effective soil resource acquisition mechanisms. Therefore, the assessment we made on its root traits, i.e., nodulation and mycorrhization, was important to better understand its agronomic requirement. Particularly, understanding its association with arbuscular mycorrhizal fungi (AMF) is very important [23, 24]. Based on our results, we report for the first time that *T. abyssinica* is N-fixing and arbuscular mycorrhizal at least at the seedling stage. Mature individuals in the filed were

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observed to be without nodules. The fact that the species is a nodulating species is to be expected. This is because, its roots were found to produce isoflavonoides [7], a biochemical required for nodulation and typical of thePapilionoideae subfamily [25]. The nodule number we record for seedlings grown on Mojo was not significantly (p<0.05) greater than the nodule number recorded for seedlings grown on soil Addis soil. However, seedlings grown on the Addis Ababa soil were not colonized by AMF. Hence, the significantly (p<0.05) higher growth recorded for*T. abyssinica* seedlings on the Mojo soil and the opposite on the Addis Ababa soil could indicate that *T. abyssinica* forms association with a selected AMF communities that are lacking in the Addis Ababa soil [26]. Rhizobia (nodulation) could have no or less significant role in this regard.

5. CONCLUSION

One of our objectives was to determine the distribution and abundance of *T. abyysinica*, a critically endangered plant species, in Shewa floristic region, Ethiopia. The other objective was to asses the agronomic requirement of the species. We have fulfilled both our objectives. From the nine locations where exploration was carried out, *T. abyssinica* populations were found only in the two, viz., Lemen and Mojo. The abundance of *T. abyssinica* was much greater than what we initially expected. However, the treat levels that were primarily used to designate its conservation status seem to persist. Hence, to improve the conservation status of the species, integrated conservation program by way of ex situ conservation, in situ conservation, and most importantly, cultivation is crucial. Based on the mesh-house experiment results, and relevant data we gathered, *T. abyssinica* could be cultivated in leptosol and degraded vertisol sites with slightly acidic to basic pH. We report for the first time that *T. abyssinica* is N-fixing and

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arbuscular mycorrhizal. Arbuscular mycorrhizal fungi (AMF) couldplay key role in the future conservation/cultivation program of the species. Hence, the AMF communities associated with *T. abyssinica* should be identified.

CONSENT: It is not applicable.

ETHICAL APPROVAL: It is not applicable.

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