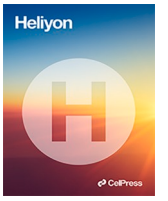
Heliyon 10 (2024) e36838

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Extraction, identification and mass production of arbuscular mycorrhizal fungi (AMF) from faba bean (*Vicia faba* L.) rhizosphere soils using maize (*Zea mays* L.) as a host plant

Zewdineh Firdu *, Getu Dida

Madda Walabu University, College of Natural and Computational Sciences, Department of Biology, Bale Robe, Ethiopia, P.O.BOX: 247

ARTICLE INFO

Keywords:
Harvesting period
Importance value
Multiplication
Root colonization
Spore density

ABSTRACT

Ethiopia is the second-largest grower of faba bean in the world next to China. The crop is highly useful with its edible seed serving as an essential protein complement of the Ethiopian diet, especially for those who cannot afford animal protein. Even though faba bean is mycotrophic to Arbuscular Mycorrhizal fungi (AMF), the different genera and species that are associated with the crop are not determined in yet at the maturity time of the crop (harvesting period). Sixteen faba bean rhizospheric soils were collected to isolate and identify AMF. Spores were extracted using the wet-sieving and decanting method. The *Glomus* genera was the most dominant, followed by the *Acaulospora* and *Gigaspora* genera. The highest spore load per 100 g of soil was observed in the sample that contained the lowest soil phosphorus. Furthermore, an inverse relationship between the spore load and soil phosphorus was observed. Three treatments were considered for mass multiplication of AMF, viz, Treatment (1) *Glomus aggregatum,* treatment (2) *Glomus* sp.BZ, and treatment *(3) Glomus* sp.AZ. However, the highest number of AMF's spore and root colonization was seen in treatment 3 with significant difference ($P < 0.05$) from the others. In conclusion, AMF constituted an important component of the faba bean rhizosphere during its harvesting period (dry season) and its multiplication using maize favored the viability and infectivity of the fungi.

1. Introduction

Faba bean (*Vicia faba* L.) is used as human food in developing countries and forages for animals in developed countries. The crop is utilized as a green vegetable or dried, fresh or canned food in the Middle East, Mediterranea region, China, and Ethiopia [1]. Ethiopia is the second largest grower of faba bean in the world next to China [2]. The crop is highly useful with its edible seed serving as an essential protein complement of the Ethiopian diet, especially for those who cannot afford animal protein [3]. Farmers in the country mostly cultivate faba bean in rotation with cereals such as wheat in different parts of the country [4].

Nutritionally, faba bean contains proteins (25 %), carbohydrates (55 %), and fat (10 %) [5]. It is also useful for human health due to its role in lowering plasma low-density Lipoproteins (LDL)-cholesterol levels due to the presence of several fibers, vitamins, and minerals within it [6]. According to Ref. [7], faba bean provides great economic and agronomic interest due to the high nutritional value of its seeds, which are rich in protein and starch. It is also considered as a versatile crop because it can be used for human consumption of its dry and fresh seeds and immature pods, for animal feed, as well as for industrial processing of dry seeds to extract

* Corresponding author.
E-mail addresses: firezew@gmail.com (Z. Firdu), Getech568@gmail.com (G. Dida).

https://doi.org/10.1016/j.heliyon.2024.e36838
Received 30 January 2024; Received in revised form 21 August 2024; Accepted 22 August 2024

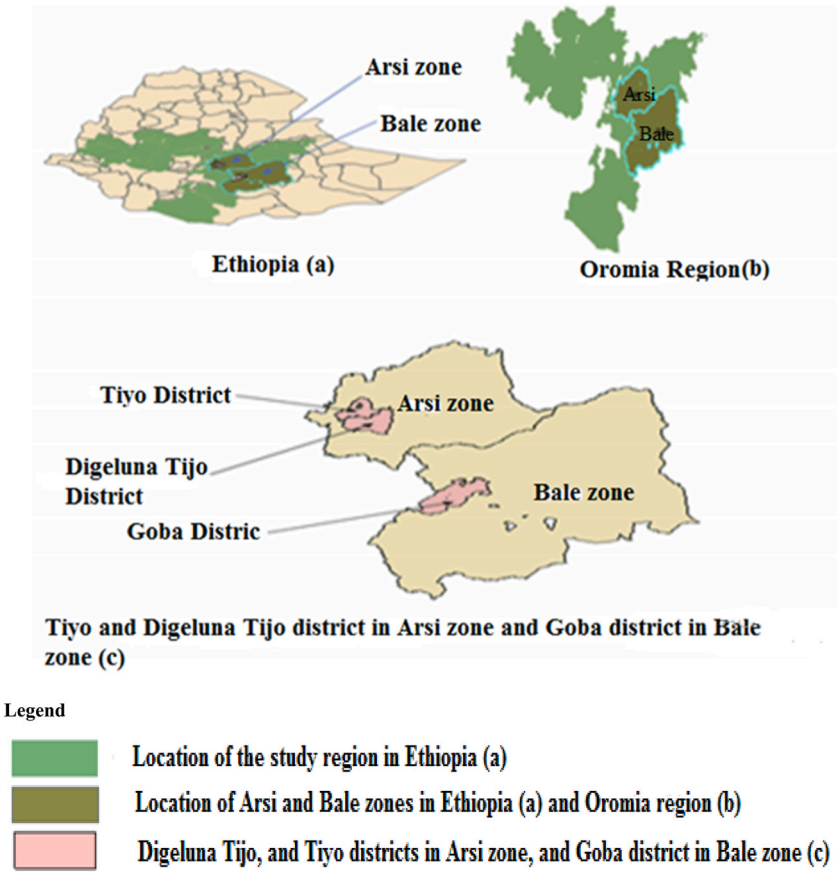


Fig. 1. A map showing the study zones and selected districts [18].

protein and produce flour.

Mycorrhizal fungi are species of fungi that intimately associate with plant roots, forming a symbiotic relationship with the plants. They are obligate biotrophs and ingest plant photosynthetic products and lipids to sustain their life cycle [8]. Arbuscular Mycorrhizal fungi (AMF) or Vesicular-Arbuscular Mycorrhizal (VAM) fungi, belonging to the phylum Glomeromycota, are symbionts with terrestrial plant roots through root colonization and spore formation [9].

Arbuscular mycorrhizal fungi (AMF), which belong to the sub-phylum Glomeromycotina, can form symbioses with 80 % of terrestrial plant species, and can positively affect various physiological and ecological processes of hosts, e.g., increased host tolerance to acidic soil [10]. These fungi have beneficial effects on the host plants, including faba beans, such as an improvement of water and nutrients absorption from the soil, especially the immobile phosphorus; mycelium from mycorrhizal plant roots can increase root size, allowing access to a greater volume of soil [11]. AMF can contribute to improving soil structure, the plant's systematic resistance responses against pathogens, and abiotic stresses [12].

Faba bean is known to associate with *Rhizobium* for biological nitrogen fixation (BNF). It appears to depend on the mycorrhiza for the absorption of water, nutrients, and minerals. Moreover, AMF can stimulate the growth of other microorganisms in the rhizosphere and alter the structure of the microbial community, influencing several biological processes, including biological nitrogen fixation [13]. Arbuscular mycorrhizal symbiosis can improve root nodulation and N_2 fixation by influencing Legume-*Rhizobium* symbiosis [14]. The enhancement of nitrogen fixation of faba bean could be attributed to AMF facilitating the mobilization of certain elements such as P, Fe, K and other minerals involved in the synthesis of nitrogenase and leghemoglobin.

Based on the aforementioned evidences, the isolation and mass multiplication of AMF associated with faba bean crop has captured our attention under greenhouse conditions using a suitable host plant. In these perspectives, there are many techniques that have been developed in the past few years for the mass production of AMF. The main difficulty behind the mass production techniques is the obligatory nature (bio-tropic) properties of the AMF, and the species level identification is not possible at early stage of its development. Currently, in vitro cultivation methods such as hydroponic system and root organ culture have been widely used for mass production of AM fungi. But the traditional method, mass production in soil-based media and living host plant is very popular and economical for the rapid production of the AM fungi [15]. From living host plants, monocots and plants with extensive root systems are very good hosts for the propagation of AM fungi [16].

At the maturity (harvesting period), the different genera and species of AMF that are associated with the faba bean are not

determined yet. Therefore, the purpose of this study was to extract the arbuscular mycorrhizal fungi spores from the faba bean rhizosphere soil samples during the harvesting period and evaluate its propagation using maize as a host plant under greenhouse conditions. Maize was selected as the host plant in this study, based on its potential to enhance AMF spore multiplication and colonization percentage. Mass multiplication of AMF spores using a suitable host plant for the production of AMF inoculum provides several benefits, which include the production of a low-cost, viable inoculum; it is eco-friendly and could easily enhance the characterization of the AMF inoculum [16,17].

2. Materials and methods

2.1. Description of the study area

The rhizosphere soil samples were collected from the Arsi zone (DigelunaTijo, and Tiyo districts) and Bale zone (Goba district) as presented below (Fig. 1) during the dry season, faba bean mowing period (November–December 2016).

2.2. Sample collection and processing

The rhizosphere soil and roots samples were collected from the study zones during the mowing period of faba bean at the dry season. The rhizosphere soil and roots were collected from separately grown faba bean plants within farm land. Five separate rhizosphere soil samples were collected per site (location), composited together in polyethylene bags and transported to the laboratory. A total of 1 kg rhizosphere soil samples were collected from standing faba beans at a depth of 10–30 cm, air dried at room temperature, used for spore extraction and physico-chemical analysis. Faba beans were uprooted, root samples were made free of soil, washed with tap water, and preserved in 60 % alcohol to maintain its structure till laboratory processing.

2.3. Soil nutrient analysis

The total nitrogen (%) content was analyzed by the Kjeldahl method as detailed by Ref. [19] with slight modifications. In this method, 3 g of soil were weighed and transferred into a distillation flask, 100 mL of distilled water was added, and 50 mL of freshly prepared 0.32 % $KMnO_4$ and 50 mL of 2.5 % NaOH were added to the soil in the distillation flash. A 100 mL beaker containing approximately 110 mL of 4 % boric acid with a double indicator was kept below the delivery end of the condenser in the distillation set. The contents were distilled at a steady rate and the liberated ammonia was collected using boric acid. The distillation was continued until the release of free ammonia and about 30 mL of distillate was collected. The ammonia collected in boric acid was titrated against 10 N H_2SO_4. The amount of nitrogen present in the sample was expressed in percentage (% N) using the following formula;

$$\% \text{ Nitrogen} = \frac{(\text{mL standard acid - mL blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample (g)}}$$

Where, the value 1.4007 stands for the Milliequivalent weight of nitrogen x100.

Soil available phosphorus (PPM) was estimated by the Bray's II method [20]. In this method, 2 g of soil was placed in a 100 mL flat-bottomed flask, 20 mL of extracting solution (0.03 M NH_4F and 0.1 M HCl) was added, shaked for 1 min and filtered in a 100 mL conical flask. A 5 mL of aliquot was then transferred in to a 50 mL volumetric flask, 5 mL of ammonium molybdate was added, and the volume was made up to $^2/_3$ by adding distilled water. A 0.25 mL of Stannous chloride was added, shaked and the absorbance was read at 660 nm spectrophotometrically and values were converted to PPM [21].

Soil organic matter (SOM) was determined according to the Walkley-Black (WB) method as described by the [22] procedure. In this procedure, about 0.5 g of finely ground soil samples were digested by 0.2 M $K_2Cr_2O_7$ solution and concentrated in H_2SO_4. After 30 min of digestion, the mixture was treated with 85 % Orthophosphoric acid. The samples were then titrated against ferrous ammonium sulfate in the presence of a diphenylamine indicator. The SOM content was calculated through multiplying the organic carbon (C) content of the soil sample by the Van Bemmelen factor (1.724).

2.4. Extraction and quantification of AMF spores

The spores of AMF were extracted by the wet-sieving and decanting method [23]. In the method, 100 g of soil sample was solubilized in 1000 mL tap water and allowed to settle for 1 min and the soil suspension was then sieved through stacked sieves having pore size of 45 μm,100 μm, 220 μm, and 500 μm. Spores from the bottom sieve (45 μm) and middle (100 μm, 220 μm) were then collected on a Petri dish measuring 85 mm in diameter and the count was performed under stereomicroscope (Wetzlar, Germany). Spores were then picked, fixed on clean glass slides in polyvinyl lacto-glycerol (PVLG) and Melzer reagent (1:1 ratio) and photographed under compound microscope (OLYMPUS-BX51, Germany). The total number of spores per 100 g of soil sample was quantified following the guidelines of International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu/cultures/cultsearch.htm).

2.5. Determination of faba bean (Vicia faba L.) root colonization by AMF

The root colonization percentage (% RCL) was performed according to the Ink and Vinegar method [24]. In the method, segments

of 0.5 cm length of root samples were treated with 10 % potassium hydroxide (KOH) solution, autoclaved at 121 °C for 10 min and rinsed several times with tap water. The cleared roots were boiled for 3 min in a 5 % ink-vinegar solution with pure white household vinegar (5 % acetic acid). Roots were de-stained by rinsing in tap water (acidified with a few drops of vinegar) for 20 min. The presence of arbuscules, vesicles, and hyphae were checked through compound microscope (OLYMPUS-BX51, Germany) to estimate the AMF colonization status of faba bean roots. A total of 50 root sample segments were examined for % RCL using the following formula:

% RCL=(Number of AMF positive faba bean root segments x100)/(Total number of faba bean root segments examined)

2.6. Morphological identification of AMF

The AMF characteristics (wall ornamentation, subtending hyphae and mode of attachment, colour and shape) were utilized for the identification of AMF spores at genera level by visiting "http://invam.caf.wvu.edu/cultures/cultsearch.htm", which provides the description of AMF and based on the different taxonomic keys proposed by different workers [25]. Some of the representative spores were recorded and documented in the form of photographs.

2.7. Diversity indices of the dominant AM fungi

In this study, the dominant genera were determined by their frequency (F), relative abundance (RA), and importance value (IV) according to Ref. [26] and the spore density (SD) was also calculated according to Ref. [27].

F=(The frequency of AM fungal genera occurred)/(Total number of AM fungal genera found in this study)* 100 %

RA = Spores number of AM fungal genera/ Total quantity of AM fungal spores' * 100 %

IV= (F + RA)/ 2 *100 %

SD = spore number of all AM fungal genera/100 g air dried soil samples

2.8. Experimental design and mass production of the predominant AMF genera

The completely randomized bock design (CRBD) was employed using 3 treatments, namely, (1) *Glomus aggregatum*, (2) *Glomus* sp. BZ, and (3) *Glomus* sp.AZ considering three replications for each treatment. In this experiment, the top 3 a.m. fungal genera/species that were found dominant in spore number and % RCL was used for mass production using maize (*Zea mays* L) under greenhouse conditions according to Ref. [28]. Maize seeds were washed under tap water, surface sterilized by 3 % sodium hypochlorite for 1 min, and rinsed in sterilized-distilled water thoroughly. Light quality and irradiant plastic pots (20 cm diameter and 9 cm internal depth/height measurement) were half filled with sterilized soil collected from faba bean rhizospheres and sterilized sand (3:1 ratio) mixture, kept in greenhouse and followed by watering for three days. Holes were made using a sterilized glass road and 40–50 spores having identical morphology were inoculated before maize seed sowing. Four surface sterilized maize seeds were sowed into pots and followed by occasional watering for three months. Part of the maize seedling root was uprooted and examined for % RCL on 30, 60, 90, and 120th days after inoculation (DAI). At the end of the 90th DAI maize plants were chopped into their stems and exposed to de-watering for one extra month. After 120th DAI, the amount of AMF spore 100 g^{-1} of soil was determined according to Ref. [23] and % RCL as outlined above [24]. A half strength of Hoagland's solution II (20 mL) was used as a nutrient with a reduced amount of phosphorus to enhance the AMF spore multiplication and colonization by the maize plant [17,29]. However, the controls were applied with tap water only.

2.9. Data analysis

The data were analyzed using a Two-way ANOVA in SPSS version 25. The comparison among means were determined by Tukey's honestly significant difference (HSD) at $\alpha = 0.05$ with a confidence interval (CI) of 95 %. The correlation among soil nitrogen (SN), soil phosphorus (SP), soil organic matter (SOM), soil organic carbon (SOC), soil pH, percentage of root colonization (PRCL), and AMF spore load (AMFSL) was conducted by Pearson's correlation analysis. Values were considered statistically significant at $P < 0.05$ throughout the analysis.

3. Results and discussion

3.1. Spore density and faba bean root colonization characteristics of AMF

In the present study, the distribution of AMF associated with faba bean rhizosphere soil samples collected from three districts (Tiyo, Digeluna Tijo, and Goba) were investigated. The result revealed variation from district to district as shown below (Table 1). In this

Table 1
Arbuscular mycorrhizal fungal spore per 100 g of faba bean rhizosphere soil sample and percentage of root colonization, Oromia Region, Ethiopia.

Area's Name	Sample code	District	SD 100 g−1 air dried soil samples	% RCL	AMF structure		
					A	H	V
Sagure	FRSS-01	Digeluna Tijo	108.38 ± 0.23^{c}	47.33 ± 0.77^{ab}	+	+	+
Sherofta Andinat	FRSS-02	Tiyo	81.88 ± 1.04^{de}	41.33 ± 0.02^{bc}	–	+	+
Burkitu Alkessa	FRSS-03	Digeluna Tijo	129.50 ± 1.12^{b}	52.67 ± 1.06^{a}	+	+	+
Asella	FRSS-04	Tiyo	79.48 ± 1.51^{de}	32.00 ± 1.00^{cd}	+	+	+
Beriti	FRSS-05	Tiyo	$84.29 \pm 0.17^{c-e}$	34.66 ± 2.06^{cd}	–	+	+
Tijo	FRSS-06	Digeluna Tijo	$86.70 \pm 0.23^{c-e}$	35.30 ± 2.31^{cd}	+	+	+
Digelu	FRSS-07	Digeluna Tijo	89.11 ± 0.34^{cd}	39.33 ± 1.06^{bc}	+	+	+
Kogo	FRSS-08	Digeluna Tijo	156.18 ± 2.90^{a}	52.67 ± 1.16^{a}	+	+	+
Sherofta	FRSS-09	Tiyo	84.29 ± 0.04^{cde}	27.32 ± 0.06^{d}	+	+	+
Molle	FRSS-10	Digeluna Tijo	69.84 ± 0.34^{de}	37.30 ± 0.06^{c}	–	+	+
Shalla	FRSS-11	Tiyo	132.46 ± 1.69^{b}	54.60 ± 1.15^{a}	+	+	+
Idiget	FRSS-12	Digeluna Tijo	$86.70 \pm 0.23^{c-e}$	40.00 ± 1.00^{bc}	+	+	+
Kechema Shuka	FRSS-13	Tiyo	65.03 ± 1.51^{de}	51.33 ± 1.15^{a}	+	+	+
Akiya	FRSS-14	Tiyo	$84.29 \pm 1.04^{c-e}$	38.61 ± 2.62^{bc}	+	+	+
Alloshe Tilo 1	FRSS-15	Goba	79.48 ± 0.23^{de}	42.00 ± 0.00^{bc}	–	+	+
Alloshe Tilo 2	FRSS-16	Goba	62.62 ± 1.04^{e}	38.66 ± 1.15^{bc}	+	+	+
CV			0.1	0.3	-	-	-

AMF- arbuscular mycorrhizal fungi, % RCL-percent of root colonization, A-arbuscules, V-vesicles, H-Hyphae, (+/−)- present/absent, SD-spore density, FRSS-faba bean rhizosphere soil sample along with their corresponding number ranging from 1 to 16 and CV-coefficient of variations. Mean values that are obtained from five replicates in the same column and followed by the same letter do not differ significantly ($P > 0.05$) by Tukey HSD analysis of Two-Way ANOVA.

regard, the spore density/load that varied from 62.62 to 156.18 was obtained from 100 g of soil samples. The maximum number of AMF spore load was found in sample FRSS-08 (156.18) followed by FRSS-11 (132.46) and FRSS-03 (129.50) at the time of faba bean harvesting. Furthermore, the percentage of AMF root colonization (% RCL) that varied from 27.32 to 54.60 % was observed in faba bean root samples examined. This indicates the presence of variation in spore densities and % RCL in the soil and root samples respectively. This might be attributed to the, edaphic characteristics and/or anthropogenic factors such as the application of fertilizer, pesticides, tillage, and monocultures) associated with soils. These factors have been reported to affect both the structures and assemblage of AMF communities in the agro-ecosystems [30,31]. In addition, edaphic characteristics such as soil pH, phosphorus level, salinity, soil disturbance, vegetation and hydrologic condition of the soil can also affect the pattern of AMF distribution and colonization of plants [32]. Furthermore, the abundance pattern of various AMF is known to vary depending on the host species, as well as the soil characteristics such as pH and texture [33]. Factors such as elevation, relative air humidity, soil pH, and available soil phosphorus (P), potassium (K), and magnesium (Mg) can also influence the AMF spore production and root colonization in plants [34].

Structurally, the hyphae and vesicles were found in all the root samples analyzed in this study (100 %), even though arbuscules were not found in sample FRSS-02, FRSS-05, FRSS-10, and FRSS-15. This might be associated with the damage that might be caused when sampling, processing (preservation) of the root samples in the present study and/or the natural morphology of the arbuscules. Beside this, the age or the maturity level of the plant can also determine the availability of arbuscules in the host. In this study, the sampled plants (faba beans) were at the age of harvesting (matured), just completing its life cycle and this might be the reason for the absence of arbuscules in the examined root samples. On the other hand, an increment of the soil nutrient content can influence the formation of arbuscules, that is a highly fertilized soil can have low number of arbuscules and vice versa. In this study, the arbuscules were absent in soil samples that were found to have relatively maximum phosphorus content as compared to the other soil samples (Table 3). AMF from the fertilized soil produces fewer hyphae, arbuscules and as many vesicles as fungi from unfertilized soil [35]. Formation and development of AMF arbuscules structures possibly depends on the hosting plant's conditions, fungal species involved and environmental conditions [36].

3.2. The diversity indices and morpho-identification of AMF associated with faba bean (Vicia faba L.)

In the present study, AMF were identified based on their morphological characteristics from soil samples collected faba bean rhizosphere in the Arsi and Bale zones (Table 2). During the identification process, spores were crushed under a compound microscope to clearly observe the structure of the spore. Five genera of AMF, namely, *Glomus*, *Acaulospora*, *Gigaspora*, *Rhizophagus,* and *Scutellospora* were identified in the present study. The dominant arbuscular mycorrhizal fungi that found were the genera of *Glomus* (56.25 %), followed by *Acaulospora* (18.75 %) and *Gigaspora* (15.63 %).

Glomus spp. were found in all soil samples except in sample FRSS-13 collected from Tiyo district. This indicated that the genera were more diversified than the other genera obtained in this study. The predominance of Glomeraceae in the rhizosphere soil of faba bean is in accordance with results of others previous studies. For example [37], confirmed the dominance of the genus *Glomus* in arid and semiarid regions. This could be explained by the remarkable adaptation of this genus to drastic conditions such as drought and salinity [38]. In this aspect, it is suggested that the genus *Glomus* exhibits a higher sporulation rate, that can enable them to recover more rapidly and could be better adapted to severe conditions [39]. In the same manner [40], reported *Glomus* genera as the dominant

Table 2
The distribution, diversity indexes and Identification of arbuscular mycorrhizal fungi associated with faba bean rhizosphere soil samples collected from Arsi and Bale zones during the dry season (November–December 2016).

Area's Name	Sample	Zone	District	Identified AMF	F (%)	RA (%)	IV (%)
Sagure	FRSS-01	Arsi	Digeluna Tijo	*Acaulospora scrobiculata*	25	3.37	14.19
				Glomus sp.	50	4.98	27.49
Sherofta Andinat	FRSS-02	Arsi	Tiyo	*Glomus* sp.	75	5.02	40.01
				Acaulospora foveata	25	1.51	13.26
Burkitu Alkessa	FRSS-03	Arsi	Digeluna Tijo	*Rhizophagus fasiculatus*	25	3.37	14.19
				Glomus hoi	50	4.98	27.49
				Glomus aggregatum	100	5.02	52.51
Asella	FRSS-04	Arsi	Tiyo	*Glomus* sp.	25	3.37	14.19
Beriti	FRSS-05	Arsi	Tiyo	*Scultellospora* sp.	20	1.51	10.76
				Glomus sp.	100	21.51	60.76
Tijo	FRSS-06	Arsi	Digeluna Tijo	*Glomus ambisporum*	25	3.37	14.19
				Acaulospora scrobiculata	25	1.51	13.26
Digelu	FRSS-07	Arsi	Digeluna Tijo	*Glomus* sp.	75	5.02	40.01
				Gigaspora rosea	25	3.37	14.19
				Glomus macrocarpum	25	1.20	13.10
Kogo	FRSS-08	Arsi	Digeluna Tijo	*Glomus* sp.	100	4.98	52.49
Sherofta	FRSS-09	Arsi	Tiyo	*Scultellospora coraloidea*	25	3.37	14.19
				Gigaspora rosea	50	4.98	27.49
Molle	FRSS-10	Arsi	Digeluna Tijo	*Gigaspora rosea*	25	0.94	12.97
				Acaulospora scrobiculata	25	3.37	14.19
Shalla	FRSS-11	Arsi	Tiyo	*Glomus geosporum*	75	5.02	40.01
				Scutellospora persica	75	5.02	40.01
				Glomus albidum	25	3.37	14.19
				Glomus sp.	75	5.02	40.01
Idiget	FRSS-12	Arsi	Digeluna Tijo	*Acaulospora foveata*	75	5.02	40.01
				Acaulospora scrobiculata	75	5.02	40.01
Kechema Shuka	FRSS-13	Arsi	Tiyo	*Gigaspora rosea*	25	1.20	13.10
Akiya	FRSS-14	Arsi	Tiyo	*Glomus* sp.	25	3.37	14.19
				Glomus macrocarpum	50	4.98	27.49
Alloshe Tilo 1	FRSS-15	Bale	Goba	*Glomus gerdemanni*	75	5.02	40.01
Alloshe Tilo 2	FRSS-16	Bale	Goba	*Gigaspora rosea*	25	1.51	13.26
				Glomus sp.	25	3.37	14.19

F-frequency, **RA**-relative abundance, **IV**- importance value, and **FRSS**-faba bean rhizosphere soil sample along with their corresponding number ranging from 1 to 16.

Table 3
A nutrient analysis of faba bean rhizosphere soil samples collected from Arsi and Bale Zones, Oromia Region, Ethiopia.

Area's Name	Sample	District	SD 100 g−1 air dried soil	Soil properties				
				% N	P (PPM)	% SOM	% SOC	pH
Sagure	FRSS-01	Digeluna Tijo	108.38 ± 0.23^{c}	0.70^{a}	$21.7^{a\text{-}c}$	3.87^{e}	4.0^{bc}	5.5^{a}
Sherofta Andinat	FRSS-02	Tiyo	81.88 ± 1.04^{de}	$0.33^{e\text{-}g}$	$19.9^{a\text{-}c}$	4.8^{e}	3.6^{bc}	3.0^{d}
Burkitu Alkessa	FRSS-03	Digeluna Tijo	129.50 ± 1.12^{b}	0.30^{fg}	14.8^{c}	7.8^{a}	5.7^{a}	6.0^{a}
Asella	FRSS-04	Tiyo	79.48 ± 1.51^{de}	0.56^{b}	$22.2^{a\text{-}c}$	6.2^{ab}	3.4^{bc}	4.1^{bc}
Beriti	FRSS-05	Tiyo	$84.29 \pm 0.17^{c\text{-}e}$	0.46^{b}	26.1^{a}	6.3^{ab}	3.7^{bc}	5.7^{a}
Tijo	FRSS-06	Digeluna Tijo	$86.70 \pm 0.23^{c\text{-}e}$	0.26^{gh}	$22.8^{a\text{-}c}$	6.0^{c}	3.7^{bc}	5.6^{a}
Digelu	FRSS-07	Digeluna Tijo	89.11 ± 0.34^{cd}	$0.33^{e\text{-}g}$	26.7^{a}	6.6^{b}	4.3^{bc}	5.3^{ab}
Kogo	FRSS-08	Digeluna Tijo	156.18 ± 2.90^{a}	$0.40^{c\text{-}e}$	11.1^{c}	7.3^{a}	4.6^{b}	6.2^{a}
Sherofta	FRSS-09	Tiyo	$84.29 \pm 0.04^{c\text{-}e}$	$0.37^{d\text{-}f}$	$19.9^{a\text{-}c}$	6.5^{ab}	3.6^{bc}	5.4^{b}
Molle	FRSS-10	Digeluna Tijo	69.84 ± 0.34^{de}	$0.37^{d\text{-}f}$	24.6^{ab}	5.0^{de}	3.1^{d}	5.4^{b}
Shalla	FRSS-11	Tiyo	132.46 ± 1.69^{b}	0.21^{i}	15.3^{c}	7.5^{a}	5.1^{a}	5.9^{a}
Idiget	FRSS-12	Digeluna Tijo	$86.70 \pm 0.23^{c\text{-}e}$	$0.32^{e\text{-}g}$	$19.1^{a\text{-}c}$	6.3^{ab}	3.5^{bc}	5.1^{b}
Kechema Shuka	FRSS-13	Tiyo	65.03 ± 1.51^{de}	0.21^{i}	26.2^{a}	4.0^{f}	2.5^{d}	5.5^{a}
Akiya	FRSS-14	Tiyo	$84.29 \pm 1.04^{c\text{-}e}$	$0.34^{e\text{-}g}$	16.5^{bc}	4.7^{e}	3.3^{de}	5.4^{b}
Alloshe Tilo 1	FRSS-15	Goba	79.48 ± 0.23^{de}	0.45^{cd}	$18.9^{a\text{-}c}$	5.3^{d}	2.6^{e}	5.2^{b}
Alloshe Tilo 2	FRSS-16	Goba	62.62 ± 1.04^{e}	0.61^{b}	$21.4^{a\text{-}c}$	6.4^{ab}	3.2^{de}	4.0^{c}

N-Nitrogen, **P**-phosphorus, **SOM**-soil organic matter, **SOC**-soil organic carbon, **SD**-spore density, **PPM**-parts per million, **pH**-soil acidity/alkalinity and **FRSS**-faba bean rhizosphere soil sample along with their corresponding number ranging from 1 to 16. Mean values in the same column that are represented by the same letter (s) are not significantly different ($P < 0.05$) by Tukey HSD analysis of One-Way ANOVA.

one in two legume crops (i.e. mung bean and soybean). The greater number of *Glomus* spores in the rhizosphere is due to easy adaptation and can produce numerous spores and easy germination in less time, i.e., only 4–6 days [41].

In this study, the frequency (F), relative abundance (RA), and importance value (IV) were determined (Table 2). The diversity

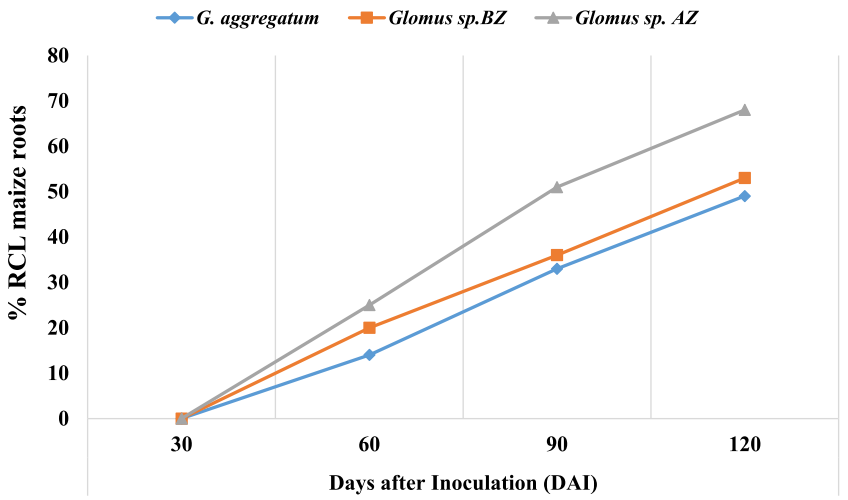


Fig. 2. Root colonization percentage (% RCL) of Arbuscular mycorrhizal fungi (AMF) mass multiplied using maize (*Zea mays* L.) as host plant under greenhouse conditions.

indexes showed uneven distributions and varying patterns of root colonization intensities of AMF in faba bean. Our data indicated that 25–100 %, 0.94–21.51 %, 10.76–60.76 of F, RA, and IV of AMF genera in the studied samples, respectively. The highest F was shown by *Glomus aggregatum* showing a value of 100.00 in sample FRSS-03 obtained from Burkitu Alkessa of Arsi zone, Digeluna Tijo district, and *Glomus* species in the samples FRSS-05, and FRSS-08. This is in agreement with the finding of 11.1–100 % reported by Ref. [42]. Furthermore, the highest RA, and IV was observed by *Glomus* species in the sample FRSS-05.

3.3. Soil nutrient analysis and its correlation with AMF spore load

The faba bean rhizosphere soil samples collected from the Arsi and Bale zone were chemically analyzed for their total nitrogen (N), available phosphorus (P), soil organic matter (SOM), soil organic carbon (SOC) and pH. The soil analysis indicated that a 0.2–0.61 %, 16.4–26.1 ppm, 4.0–7.8 %, 2.5–5.1 %, and 5.1–6.2 variation of total N, available P, SOM, SOC and pH respectively as presented below (Table 3).

Phosphorus deficiency is a significant limiting factor for the growth and yield of crops that affects approximately 50 % of all agricultural ecosystems around the world [32]. Our data showed that the highest (156.18 ± 2.90) spore load of AMF 100 g^{-1} of soil sample that contained the lowest phosphorus content (11.1 ppm). Similarly [43], have also reported greater mycorrhizal colonization (74.32 %) of AMF in the Chirikyaku areas where less phosphorus was found in the soil samples (3.48 ppm). This might be due to the need of plants to associate with phyto-beneficial microorganisms such as AMF that take part in nutrient solubilization and transportation for plants. In contrast, the amount of SOM, and SOC was the highest in the sample that contained the highest AMF spore load, indicating the direct relationship of the AMF spore. This might be due to the transportation of solubilized nutrients in the soil due to the presence of arbuscular mycorrhizal fungi with the hosting plants of this study. On top of significantly aiding the phosphorus supply to plants, AMF can help plants acquire macronutrients and micronutrients like Cu, K, Mg, N, and Zn, especially when they're present in less soluble forms in soils [44]. Arbuscular mycorrhizal fungi (AMF) enhance the root zone absorption area by 10–100 % and improve the plant's ability to utilize more soil resources [45].

The Pearson's correlation analysis revealed a negative relationship ($r = -0.753^{**}$) between AMF spore load (AMFSL) and phosphorus that was available in the soil samples. This is an indicator for the inhibitory activity of phosphorus at its highest concentration on mycorrhizal fungi sporulation and vice versa in crops. In relation to phosphorus, there are studies that report an increase in mycorrhizal colonization with low levels of phosphorus content in soils [46]. However, the sporulation of AMF was found to correlate positively with the soil pH determined in this study ($r = 0.782^{**}$), showing an increment of AMF as the soil acidity increased and vice versa. Similar finding was reported by Ref. [47] where by mycorrhization value of 50–84 % was found in areas with soil pH values that were raised from 3.5 to 4.2. Even though AMF actively alters host tolerance to acidic soil, AMF themselves can be affected by low pH in terms of their colonization and functionality [48].

Colonization and spore production are two stages of the life cycle of AM fungi. In this study, the percentage of AMF root colonization (% RCL) was also found to be correlated ($r = 0.643^{**}$) with arbuscular mycorrhizal fungi spore load (AMFSL). This is in agreement with [49], who have reported a significant positive correlation between the sporulation and root colonization of AMF in the woody vegetation species that were common in the exclosured area. However [50], have reported no correlation between AMF sporulation and root colonization in different crops, which is different from the present finding. This inconsistency might be due to the irregular spatial distributions of pores and the complex structures of roots in different host plants and the seasons of the studies. These conditions can lead to variations in the sporulation and colonization rate among different plant species [51]. In addition, there might be no obvious correlation between the spore-producing ability of AM fungi and their colonization ability in host plants, and a strong

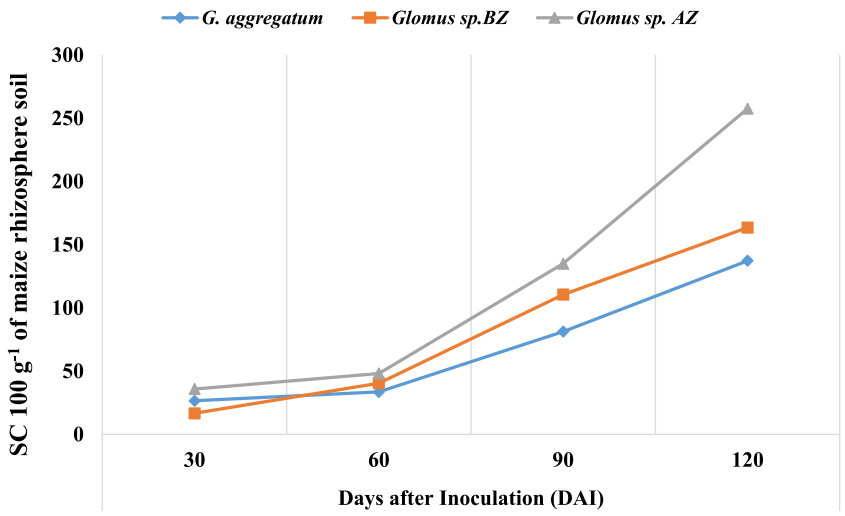


Fig. 3. Spore count (SC) of Arbuscular mycorrhizal fungi (AMF) obtained from the rhizosphere soil samples of maize (*Zea mays* L.) used to propagate AMF under greenhouse conditions.

spore-producing ability does not correlate to a strong colonization ability [50].

3.4. Mass multiplication and root colonization of the dominant AMF using maize (Zea mays L.)

The mass multiplication of AM fungi obtained dominant from faba bean rhizospheric soil samples indicated variation form treatment to treatment. In the present study, no AM fungi structures and root colonization were observed on 30 DAI in all the treatments. This might be due to the insufficiency of time for the development of AM fungi to bear the structures and the absence of stresses at a time. AM fungi form an association when the host plant is exposed to stress conditions such as nutrient scarcity and formation of depletion zone in the root zone, where the plant release a signal molecule, strigolactones through time [52,53].

The analysis using a Two-Way ANOVA indicated that the culturing period (days) was more significant in determining AM fungi structures (hyphae, vesicles and arbuscules), spore load and % RCL (Fig. 2). The formation of hyphae and arbuscules were seen starting from the 60th DAI in all the treatments, whereas the vesicles were seen starting from 90 DAI for treatment 1 (*Glomus aggregatum*) and treatment 3 (*Glomus* sp. AZ). The age of the host plant may also influence the formation of hyphae, arbuscules and vesicles. Hyphae and arbuscules were formed earlier in this study, which are beneficial to facilitate nutrient exchange between host and AM fungi.

On the other hand, the spore count was highest in treatment 3 (257.67 $\pm$ 1.69) followed by treatment 2 (163.67 $\pm$ 0.6) and treatment 1 (137.33 $\pm$ 0.5) at the end of 120 days (Fig. 3). However, the spore load of treatment 3 was found to be significantly different as compared to treatment 1 and 2 ($P < 0.05$) by 120 days. This indicated that treatment 3 had more proliferation ability using maize as a host plant and soil-sand mixture as a substrate compared to other treatments. This finding is in line with [54] where the Glomeraceae spp. was found more dominant in soil-sand mixture mass production of AM fungi than *Glomus intraradices*, *Glomus clarum* and *Glomus etunicatum* using maize as a host plant during mass production. In addition, there was a constant increment of spore load per 100 g of soil and percentage of RCL from 30 to 120 days in all the treatments. Among the three treatments, at the end of 120 days, the best result was seen in treatment 3 having the highest % RCL (68 $\pm$ 4) and sporulation (257.67 $\pm$ 1.69). The highest sporulation and root colonization that was seen in this treatment could be due to higher compatibility between the AM fungal isolates and maize [16].

Conclusion

In the present study, AMF constituted an important component of faba bean rhizosphere planted in the Arsi and Bale zones with the dominant genera of *Glomus* spp during the harvesting period of faba bean. The selection of this faba bean native AMF and its multiplication in trap culture using maize as a host plant favored the viability, and sporulation of AMF that could be used as a bio-inoculant for faba bean cultivation.

Recommendations

This study was conducted during the dry season at the time of faba bean crop harvesting in the Arsi and Bale zones in Ethiopia. Hence, to generalize the overall status of faba bean association with AMF, additional study should be conducted considering different seasons, including the wet season. In addition, the identification of AMF should be done using molecular techniques including meta genomics to reveal out the holistic status of faba bean associating AMF.

Data and code availability statement

The data will be made available on request.

CRediT authorship contribution statement

Zewdineh Firdu: Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Getu Dida:** Writing – review & editing, Writing – original draft, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Addis Ababa University, Department of Microbial, Cellular and Molecular Biology for providing all the laboratory facilities and Madda Walabu University for the financial support of this study.

References

[1] N.E. El-Wakeil, T.N. El-Sebai, Role of biofertilizer on faba bean growth, yield and its effect on bean aphid and the associated predators, Res. J. Agric. Biol. Sci. 3 (2007) 800–807.
[2] K. Edossa, C. Raghavaiah, Production potential of faba bean (*Vicia faba* L.): genotypes in relation to plant densities and phosphorus nutrition on vertisols of central highlands of West Showa Zone, Ethiopia, East Africa, Advances in Crop Science and Technology 4 (2016) 287–305.
[3] B.A. Bakry, T.A. Elewa, M.F. El karamany, M.S. Zeidan, M.M. Tawfik, Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition, World J Agricultural Sciences 7 (2011) 68–72.
[4] S. Sahile, C. Fininsa, P.K. Sakhuja, A. Seid, Effect of mixed cropping and fungicides on chocolate spot (*Botrytis fabae*) of faba bean (*Vicia faba*) in Ethiopia, Crop Protect. 27 (2008) 275–282.
[5] Grain Research and Development Corporation, Faba beans. Grown Notes, 2017. ISBN:978-1-921779-09-1.
[6] O. Olaofe, E.T. Akintayo, Prediction of isoelectric points of legume and oil seed proteins from their amino acid composition, J Techno Sci. 4 (2000) 49–53.
[7] R.D. Semba, R. Ramsing, N. Rahman, K. Kraemer, M.W. Bloem, Legumes as a sustainable source of protein in human diets, Global Food Secur. 28 (2021) 100520.
[8] Y.N. Jiang, W.X. Wang, Q.J. Xie, N. Liu, L.X. Liu, D.P. Wang, et al., Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi, Science 356 (2017) 1172–1175.
[9] L. Tedersoo, S. Sánchez-Ramírez, U. Kõljalg, M. Bahram, M. Döring, High-level classification of the fungi and a tool for evolutionary ecological analyses, Fungal Divers. 90 (2018) 135–159.
[10] J.W. Spatafora, Y. Chang, G.L. Benny, K. Lazarus, M.E. Smith, M.L. Berbee, A phylum-level phylogenetic classification of Zygomycete fungi based on genome-scale data, Mycologia 108 (2016) 1028–1046.
[11] N. Diagne, M. Ngom, P.I. Djighaly, D. Fall, V. Hocher, S. Svistoonoff, Roles of arbuscular mycorrhizal fungi on plant growth and performance: importance in biotic and abiotic stressed regulation, Diversity 12 (10) (2020) 370, https://doi.org/10.3390/d12100370.
[12] W. Weng, J. Yan, M. Zhou, X. Yao, A. Gao, C. Ma, J. Cheng, J. Ruan, Roles of arbuscular mycorrhizal fungi as a biocontrol agent in the control of plant diseases, Microorganisms 10 (7) (2022) 1266, https://doi.org/10.3390/microorganisms10071266.
[13] J.M. Freire, S. M. de Faria, J.E. Zilli, O.J. Saggin Júnior, I.S. Camargo, J.R.C. Rouws, E. da C. Jesus, Symbiotic efficiency of inoculation with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi in *Tachigali vulgaris* seedlings, Rev. Árvore 44 (2020).
[14] R. Ben-Laouane, M. Baslam, M. Ait-El-Mokhtar, M. Anli, A. Boutasknit, Y. Ait-Rahou, S. Toubali, T. Mitsui, K. Oufdou, S. Wahbi, A. Meddich, Potential of native arbuscular mycorrhizal fungi, rhizobia, and/or green compost as alfalfa (*Medicago sativa*) enhancers under salinity, Microorganisms 8 (11) (2020) 1695.
[15] S. Sharma, S. Sharma, A. Aggarwal, V. Sharma, M. Singh, S. Kaushik, Mass Multiplication of Arbuscular Mycorrhizal Fungi, Mycorrhizal Fungi, 2017, pp. 155–173.
[16] S. Kuppusamy, K. Kumutha, Standardization of the substrate material for large scale production of arbuscular mycorrhizal inoculum, Intl. J. Biotech. 4 (2011) 625–631.
[17] A.F. Oluwaseun, E.A. Adenike, O.B. Olubukola, Propagation and characterization of viable arbuscular mycorrhizal fungal spores within maize plant (*Zea mays* L.), Journal of the Science of Food and Agriculture/Journal of the Science of Food and Agriculture 101 (14) (2021) 5834–5841.
[18] Z. Firdu, L. Maia, J. Teodoro, T. Alemu, F. Assefa, Characterization of faba bean (*Vicia faba* L.) rhizosphere associating rhizobacteria against *Botrytis fabae* AAUBF-12 and their plant growth-promoting properties, Heliyon (2022) e08861.
[19] J.M. Bremner, C.S. Mulvaney, Nitrogen-total, in: A.L. Page (Ed.), Methods of Soil Analysis, Part 2. Madison, WI: Am. Soc. Agron. Mon. No. 9, second ed., 1982, pp. 595–624.
[20] R.H. Bray, L.T. Kurtz, Determination of total, organic, and available forms of phosphorus in soil, Soil Sci. 59 (1945) 39–45.
[21] X. Lin, J. Zhang, H. Chen, L. Han, Determination of available phosphorus in alkaline soil by molybdenum blue spectrophotometry. IOP Conference Series, Earth and Environmental Science 781 (5) (2021) 52003, 052003.
[22] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon and organic matter, in: A.L. Page (Ed.), Methods of Soil Analysis, Part 2. Agronomy Monographs 9, ASA and SSSA, Madison, WI, 1982, pp. 539–579.
[23] J.W. Gerdemann, T.H. Nicolson, Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting, Trans. Br. Mycol. Soc. 46 (1963) 235–244.
[24] H. Vierheilig, A.P. Coughlan, U. Wyss, Y. Piche, Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi, Appl. Environ. Microbiol. 64 (1998) 5004–5007.
[25] D. Schwarzott, C. Walker, A. Schüßler, *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is non-monophyletic, Mol. Phylogenet. Evol. 21 (2001) 190–197.
[26] J. Yang, X.L. He, L.L. Zhao, Species diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Salix psammophila* in inner Mongolia desert, Biodivers. Sci. 19 (2011) 377–385.
[27] J. Wang, G.G. Wang, B. Zhang, Z. Yuan, Z. Fu, Y. Yuan, L. Zhu, S. Ma, J. Zhang, Arbuscular mycorrhizal fungi associated with tree species in a planted forest of eastern China, Forests 10 (2019) 424.

[28] M. Habte, N.W. Osorio, Arbuscular Mycorrhizas: Producing and Applying Arbuscular Mycorrhizal inoculum. Honolulu (HI), University of Hawaii, 2001, pp. 1–47.
[29] D.D. Douds, N.C. Schenck, Cryopreservation of spores of vesicular-arbuscular mycorrhizal fungi, New Phytol. 115 (1990) 667, 647.
[30] X. Lin, Y. Feng, H. Zhang, Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in North China revealed by 454 pyrosequencing, Environ. Sci. Technol. 6 (2012) 5764–5771.
[31] E. Verbruggen, M.G.A. van der Heijden, J.T. Weedon, G.A. Kowalchuck, W.F.M. Roling, Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils, Mol. Ecol. 12 (2012) 2341–2353.
[32] A. Jamiołkowska, A. Księżniak, A. Gałązka, B. Hetman, M. Kopacki, S. Bednarz, Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil, Intl. Agrophys 32 (2018) 133–140.
[33] L. Lara-Capistran, R. Zulueta-Rodriguez, B. MurilloAmador, D.M. Verdecia Acosta, L.G. Hernandez-Montiel, Biodiversity of AM fungi in coffee cultivated on eroded soil, Agronomy 11 (567) (2021) 1–10.
[34] C.D. Melo, C. Walker, C. Krüger, P.A.V. Borges, S. Luna, D. Mendonça, H.M.A.C. Fonseca, A.C. Machado, Environmental factors driving arbuscular mycorrhizal fungal communities associated with endemic woody plant *Picconia azorica* on native forest of Azores, Ann. Microbiol. 69 (13) (2019) 1309–1327.
[35] N.C. Johnson, Can fertilization of soil select less mutualistic mycorrhizae? Ecol. Appl. 3 (4) (1993) 749–757.
[36] X. Zhao, Arbuscular mycorrhizal and dark septate fungal associations in riparian plants of the three gorges reservoir region, Southwest China, Aquat. Bot. 133 (2016) 28–37.
[37] F. Ndoye, A. Kane, E.L. Ngonkeu Mangaptché, N. Bakhoum, A. Sanon, D.S.M.O. Diouf, E. Baudoin, K. Noba, Y. Prin, Changes in land use system and environmental factors affect arbuscular mycorrhizal fungal density and diversity, and enzyme activities in rhizospheric soils of *Acacia senegal* L wild, Ecol. 13 (2012), https://doi.org/10.5402/2012/563191.
[38] J. Błaszkowski, M. Tadych, T. Madej, Arbuscular mycorrhizal fungi (Glomales, Zygomycota) of the Błedowska desert, Poland, Acta Soc. Bot. Pol. 71 (2002) 71–85.
[39] L. Whitfield, A.J. Richards, D.L. Rimmer, Relationships between soil heavy metal concentration and mycorrhizal colonization in *Thymus polytrichus* in northern England, Mycorrhiza 14 (2004) 55–62.
[40] A. Hindumathi, B.N. Reddy, Occurrence and distribution of arbuscular mycorrhizal fungi and microbial flora in the rhizosphere soils of mung bean [*Vigna radiata* (L.) Wilczek] and soybean [*glycine max* (L.) Merr.] from Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh state, India, Adv. Biosci. Biotechnol. 2 (2011) 275–286.
[41] M. Wang, P. Jiang, Colonization and diversity of AM fungi by morphological analysis on medicinal plants in southeast China, Sci. World J. 753842 (2015) 7.
[42] C. Yang, Y. Liu, W. Zhao, N. Wang, Colonization characteristics and diversity of arbuscular mycorrhizal fungi in the rhizosphere of Iris lacteal in Songnen saline-alkaline grassland, Phyton 90 (2021) 719–729.
[43] W.F. Ríos-Ruiz, L. Barrios-López, J.C. Rojas-García, R.A. Valdez-Nuñez, Mycotrophic capacity and diversity of native arbuscular mycorrhizal fungi isolated from degraded soils, Sci. Agropecu. 10 (2019) 99–108.
[44] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, Academic press, London, 2008.
[45] H. Etesami, B.R. Jeong, B.R. Glick, Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant, Front. Plant Sci. 12 (2021) 699618.
[46] Y. Chen, J.G. Yuan, Z.Y. Yang, G.R. Xin, L. Fan, Associations between arbuscular mycorrhizal fungi and *Rhynchrelyrum repens* in abandoned quarries in southern China, Plant Soil 304 (2008) 257–266.
[47] C. Corpoica, Mycorrhizae as an Alternative to Sustainable Management of Tropical Agroecosystems, Colombia, 1998, pp. 6–27. Meta, Villavicencio.
[48] X. Liu, F. Zhang, Z. Zhao, H. Zhu, Q. Yao, Acidic soil inhibits the functionality of arbuscular mycorrhizal fungi by reducing arbuscule formation in tomato roots, Soil Sci. Plant Nutr. 66 (2) (2020) 275–284.
[49] E. Birhane, K.M. Gebremedihin, T. Tadesse, M. Hailemariam, N. Solomon, Enclosures restored the density and root colonization of arbuscular mycorrhizal fungi in Tigray, Northern Ethiopia, Ecological Processes 6 (2017) 121.
[50] C.X. Yang, S.C. Huang, F. Chen, L.L. Li, Infection characteristics and diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Inula japonica* in Songnen saline-alkaline grassland, Pratacult. Sci. 34 (2017) 231–239.
[51] Z. Zhao, Y. Xia, X. Qin, X. Li, L. Cheng, Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, Southwest China, Mycorrhiza 11 (2001) 159–162.
[52] C. Ruyter-Spira, S. Al-Babili, S. van der Krol, H. Bouwmeester, The biology of strigolactones, Trends Plant Sci. 18 (2013) 72–83.
[53] M. Lopez-Obando, Y. Ligerot, S. Bonhomme, Strigolactone biosynthesis and signaling in plant development, Dev 142 (2015) 3615–3619.
[54] K.M. Gaonkar, B.F. Rodrigues, Studies on viable inoculum production in selected *Glomeraceae* species, Advances in Plant Sciences and Biotechnology (2015) 58–63.