Vol. 19(7), pp.487-499, July, 2020
DOI: 10.5897/AJB2020.17133
Article Number: DD6079864432
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Diversity of arbuscular mycorrhizal fungi (AMF) and soils potential infectivity of *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. rhizosphere in Senegalese salt-affected soils

Samba-Mbaye, R. T.[1,2,3]*, Anoir, C. M.[1,2,3], Diouf, D.[1,2,3,4], Kane, A.[1,2,3], Diop, I.[1,2], Assigbete, K.[5], Tendeng, P.[2,3] and Sylla, S. N.[1,2]

[1]Département de Biologie Végétale, Université Cheikh Anta Diop de Dakar (UCAD), Sénégal.
[2]Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel-Air, Dakar, Sénégal.
[3]Laboratoire Mixte International Adaptation des Plantes et Microorganismes Associés aux Stress Environnementaux (LMI LAPSE), Dakar, Sénégal.
[4]UFR Environnement, Biodiversité et Développement Durable, Université Sine Saloum El Hadj Ibrahima Niass (USSEIN), BP 55, Kaolack, Sénégal.
[5]Laboratoire Mixte International - Intensification Ecologique des Sols Cultivés en Afrique de l'Ouest (LMI-IESOL), Institut de Recherche pour le Développement, Dakar, Sénégal.

Received 31 March, 2020; Accepted 14 July, 2020

***Vachellia nilotica,* a multipurpose tree useful to rural populations, is often used to rehabilitate Senegalese salt-affected soils due to its salt-tolerance probably related to arbuscular mycorrhizal fungi (AMF) symbiosis. This work aimed to determine the AMF communities associated with *V. nilotica* rhizosphere in salt-affected soils and their potential infectivity. Soils were sampled from six sites in the central region of Senegal. Soil chemical properties and total fungal communities were analyzed. Spores number was estimated and morphotypes were identified. Soil-borne AMF were trapped using *V. nilotica* plants to analyze their infectivity potential and their effect on plant growth and biomass production. Results showed the capacity of *V. nilotica* to grow in a heterogeneity of soils and revealed that rhizospheric soils are rich in AMF infectives strains. Data showed that salinity was the main factor that influenced the fungal structure and has a negative effect on AMF development, hence the highest number of spores in high salinities soils. This negative effect is greater in soils collected in dry season than in wet season. The highest density of spores was found in soils beneath the canopy, indicating the influence of plant rhizosphere on AMF diversity. Morphotypes identified are related to the genus *Glomus, Gigaspora, Acaulospora.* AMFs in these soils can establish a good mycorrhization with *V. nilotica* and increase plant biomass and height.**

Key words: *Vachellia nilotica*, arbuscular mycorrhizal fungi, diversity, salt affected soils, rhizosphere, infectivity.

INTRODUCTION

Soil salinization is a worldwide problem amplified by global warning, especially in arid and semiarid regions. Salinization reached about 7% of total areas and 20% of cultivated soils in the world (Zhu, 2001; Abbas et al., 2013).

In Senegal, about 45% of cultivated soils are affected by salinization (LADA, 2009) and more than 8% in Fatick and Kaolack, center region of the country (PAPIL, 2013). Consequences of soil salinity are harmful especially in arid lands ranging from plant diversity lost to total lack of plants (desert) including damaging soil properties and decline of fertility. One of the sustainable strategies to rehabilitate these salts affected soils and control salinity is reforestation with adapted perennial plants like leguminous. *Acacia* species such as *Vachellia nilotica* are often used in Senegal for reforestation (Sambou et al., 2010) because of their tolerance to salinity (Singh and Thomson, 1992; Giri et al., 2007). *V. nilotica* is a multipurpose tree species for rural populations: fuel-wood, timber, gum, therapeutic use (Bargali and Bargali, 2009). However, its symbiotic association with both arbuscular mycorrhizal fungi (AMF) and rhizobia could contribute to plant adaptation to harsh environments such as saline soils because they could supply nitrogen, water, phosphorus and other nutrients. AMF contributed to crop productivity and ecosystem preservation through many ecosystemic services including plant nutrition, soil structure, and plants protection against abiotic stresses by improving drought tolerance, protection against pathogens, stimulation of synthesis of plant secondary metabolites beneficial to human health (Gianinazzi et al., 2010; Hanin et al., 2016; Begum et al., 2019).

Several studies have reported beneficial effect of AMF on *Acacia* spp. plant growth and adaptation to harsh environments (Raghuwanshi and Upadhyay, 2004; Diouf et al., 2005; Ashraf et al., 2008; Fall et al., 2017; Manga et al., 2017). Thus, some previous studies also showed that *V. nilotica* is mycotrophic and inoculation with AMF can enhance plant productivity (Dommergues et al., 1999; Giri et al., 2007; Chandrasekaran et al., 2014). But few studies addressed indigenous communities of AMF associated to *V. nilotica* in salt-affected soil in this part of the country where settlements of this tree were well established. However, it is established that there is variability in the response of plants to AMF depending on the nature of the soil (Bâ et al., 1996; Diop et al., 2015). Moreover, rhizospheric soil of *V. nilotica* trees established in salt-affected soils could harbor indigenous communities of AMF that could be identified as inoculum to enhance *V. nilotica* plants growth and to contribute to their survival and their establishment in saline soils. This work aimed to describe morphological diversity of indigenous AMF associated to *V. nilotica* rhizosphere in salt affected soils and their effect on plant growth, in order to identify potential AMF inoculum strains able to optimise *V. nilotica* plant nursery development used for reforestation programs of degraded salt affected soils.

MATERIALS AND METHODS

Soils sampling and chemical properties analysis

Soils samples were collected from six sites in Fatick and Kaolack regions, center of Senegal, and their geographic positions are recorded by Global Position System (Figure 1). Climate in this area is characterized by the alternation of two seasons: a long dry season which is spread over eight months (October-June) and a short rainy season up to four months (June-October). The temperature fluctuates between 21-24°C in December-February and 35-42°C particularly in May-June (SES, 2016) and annual precipitations are about 400-800 mm.

Soils were sampled in the rhizosphere of mature *V. nilotica* trees from six salt affected sites in Fatick and Kaolack regions (Djilass, Nguessine, Fatick, Ngane, Kahone and Sadioga) to recover more areas. The same sites are sampled in both wet and dry seasons, except Nguessine 2 which was inaccessible in wet season. For each site, two samples soil mixtures were collected, beneath the canopy of *V. nilotica* (SC) and outside the canopy (as control, T). Each sample is a mixture of three samples (replicates) from three rhizospheric trees. Four soil subsamples were collected in each sample point (1 m from the trunk in all cardinal directions) at a depth of 0-25 cm and pooled to one homogeneous rhizospheric soil sample.

The chemical properties analysis of the soils samples were carried out at the LAMA lab (Laboratoire des Moyens Analytique) certified International Standard for Organization 9001, version 2000; Institut de Recherche pour le Développement, IRD-Bel Air, Dakar-Sénégal (www.lama.ird.sn)

Soils DNA extraction

DNA extraction of soil microbial communities was performed using the Power Soil DNA isolation kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. DNA extracts were stored in 50 µl of sterile water and kept cold for PCR uses later.

Nested-PCR (PCR-DGGE)

The analysis of total fungal community of soils was performed using "Nested PCR" technique. A first PCR amplification was carried out with the primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993, 1996) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The following thermocycle programme was used for the PCR amplification: 94°C (5 min) initially, followed by 30 cycles of 94°C (30 s); 57°C (30 s); 72°C (1 min) and 72°C final (10 min). PCR products were migrated in a 1% agarose gel stained with ethidium bromide and visualized in UV light.

PCR products with 700 to 900 bp length were then used as template DNA for a second PCR with the primers ITS1F-GC (Muyzer et al., 1993) and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White et al., 1990). This PCR reaction yielded DNA fragments

*Corresponding author. E-mail: ramatoulaye.mbaye@ucad.edu.sn. Tel: 221 77 449 68 63.

Figure 1. Geographical location of soils sampling sites.

length of about 400 bp with the initial PCR cycle of 94°C (5 min), followed by 30 cycles of 94°C (30 s); 60°C (30 s); 72°C (30 s) and 72°C final (10 min). The reaction mixture contained per tube, 1 µL of primers (25 µM), 0.5 µL DNA (2.5 ng / µL); Taq Ready To Go bead (Amersham Biosciences, USA) (contains 2.5 µg *Taq* DNA polymerase, 200 µM dNTP, 10 mM Tris-HCl pH 9, 50 mM KCl and 1.5 mM $MgCl_2$) for a final volume of 25 µL.

DGGE gel migration

DGGE is based on the electrophoretic separation of double stranded DNA molecules depending on the difference in their melting behaviour in a gradient of either a denaturing agent or temperature (Muyzer et al., 1993). The DGGE resolution distinguishes two different PCR bands from a single base (Muyzer and Smalla, 1998).

The rDNA fragments obtained by Nested PCR were separated on an 8% polyacrylamide gel [40% acrylamide-bisacrylamide (37.5: 1)] with a gradient of denaturants (23 to 58%) for the fungal community (100% denaturant contains 7 M urea with 40% formamide).

The migration was carried out at 100 volts for 18 h in a TAE buffer (1X) at 60°C. Visualisation of rDNA fragment profiles was done at UV (254 nm) after staining the gel with BET (1 mg / ml). Images capture of rDNA profiles was done using Bio-Capt software (Vilber Lourmat, France). Profiles were analysed with the Phoretix 1D tutorial version 10 (TotalLab Ltd) tape detection, based on presence-absence, intensity of bands. After analysis and validation, a dendrogram was generated.

Spores extraction and morphotypes identification

Spores of arbuscular mycorrhizal fungi were isolated from soil samples using the wet sieving and decanting method as described by Gerdemann and Nicolson (1963). An aliquot of 100 g of each soil sample was used. The supernatant containing the spores was filtered through superposed sieves of decreasing size (500, 200, 100 and 50 µm) in order to recover the maximum number of spores. This operation was repeated at least 3 times. All particles retained in the sieves of 200, 100 and 50 µm were recovered in 30 ml tubes containing distilled water (spores suspension). The separation of the spores was performed using two sucrose solutions, 20 and 60%. At the end, the 50 µm sieve contents were collected with tap water and stored at 4°C for preservation.

The total number spores in 100 g of soil were observed and counting under the WILD M400 binocular loupe. Microscopic observation was used to identify and to photography the different morphotypes from the rhizospheric soils of *V. nilotica* and soils outside canopy.

Morphological spore characterization was made in comparison with genus descriptions provided by the International Culture Collection of Arbuscular and Vesicular Arbuscular Fungi (http://invam.caf.wvu.edu). Criteria for morphological spore characterization were mainly based on spore size, wall colour and structure and hyphal attachment (Cho et al., 2006).

AMF trapping in greenhouse

The experiment was carried out in greenhouse using *V. nilotica* plants to compare AMF infectivity of rhizospheric soils from six sites. Seeds were first treated with concentrated H_2SO_4 and thoroughly washed with running tap water and soaked in water overnight. Two germinated seeds were transplanted in plastic bag that contained 1 kg sterilised (by autoclaving 180°C for 4 h) soil of Sangalkam, a nutrients poor sandy soil with low P content (Bâ et al., 1999). A week after, one plant was taken off to have plants with homogeneous growth.

Experiment has compared 12 treatments inoculated using six rhizospheric soil samples and their controls collected outside canopy and one non inoculated as control. Each treatment has ten replicates. Inoculation was performed by adding 50 g per bag of each rhizospheric soil sample (as inoculum) before transplanting

the seeds. Non-inoculated pots received the same amount of autoclaved soil. After three months, the plants were harvested, biomass estimated and height also measured. Roots were sampled for observation of mycorrhizal colonization and estimation of mycorhization parameters.

Roots coloring

Roots colouring was performed as described by Philips and Hayman (1970). Samples roots were thoroughly cleaned with tap water to remove soil particles and then placed in a 10% KOH solution to discolour them and to empty the cytoplasmic contents of the root cells. The tubes were boiled in a water bath for 1 h at 90°C. The roots were thoroughly rinsed with water to remove the KOH, followed by roots dewatering and lightening with bleach for 3 min. The staining of the roots was carried out in a Trypan blue solution of 0.05% and boiled in a water bath at 80°C for 30 min. The stain was removed and roots rinsed for last time to remove the excess. The roots are finally soaked in tap water.

Estimation of mycorrhizal roots colonization

The intensity and frequency of mycorrhization were determined using the Trouvelot et al. (1986) method. For each sample, the coloured roots were cut into fragments of about 1 cm each and deposed between slides and lamellae which are parallels and coated with 90% glycerol for preservation. One slide contains 15 fragments, with 5 repetitions for each treatment. Slides were observed under a microscope (x 20) fragment by fragment. The colonization level of the roots was estimated by presence of hyphae, vesicles or arbuscules in the roots.

Statistical analysis

Analysis of variance (ANOVA) was performed on all data using XLSTAT (version 2010, Addinsoft) software. Mean values of all treatments were compared using Duncan test (Honestly significant differences, HSD) at the significance level ($p<0.05$). The correlation between the chemical properties of the soils was established with the PCA test (principal component analysis) according to the type of Pearson (n).

RESULTS

Chemical properties of soils

Results showed differences in salinity rate and pH for sampling soils sites. Samples of dry season showed high soils salinity in localities of Djilass beneath canopy (SC) and outside (T), Nguessine2T, Fatick T and Nguessine1 (SC and T) and low salinity soils such as Nguessine2 SC, Sadioga (SC and T), Ngane (SC and T) and Kahone (SC and T) and Fatick SC. Comparison of different soils chemical properties using PCA analysis allowed the distinguishing of three groups of soils (Figure 2): Group 1 correspond to soils with high salinity, acidic pH and high percentage of exchangeable sodium. Soils of this group are TNguessine 1 (TNGE1), Nguessine1 (NGE1), TDjilass (TDJI), Djilass (DJI), TFatick (TFAT); Group 2 correspond to soils with low salinity and alkali pH, an abundance of exchangeable bases (K, N, Ca, C), a high rate of organic matter (% MO): soils from Kahone (KAH), Kahone control (TKAH), Fatick (FAT), Ngane control (TNGA). The group 3 grouped soils from Nguessine2 SC, Sadioga (SC and T) and Ngane SC with low salinity and acid pH, a low level of mineral elements and organic matter. This demonstrated the capacity of *V. nilotica* to grow in a heterogeneity of soils.

Total fungal richness of soils

In order to analyze how salinity affect fungal population, soil richness in fungal communities was estimated by analyzing the ITS of 18S gene region of the total DNA extracted from the rhizosphere soils sampled beneath and outside canopy of the *V. nilotica.* The DGGE profiles were compared for their similarity and the resulting dendrogram (Figure 3) showed two main groups of soils (I, II).

Group I could be divided into three subgroups (IA, IB, IC). IA was composed of soils from Kahone (beneath and outside canopy), Sadioga (beneath and outside canopy) and Ngane outside canopy characterized by low salinity and pH that can be acidic or basic. It should be noted that the samples under cover have profiles similar to those from outside the canopy, indicated that low salinity did not affect fungal communities.

Group IB contains soils of Ngane, Nguessine 1 and Nguessine 2 and Djilass all having an acidic pH, and salinity that can be low or high. The IC group with low salinity and acidic pH was composed of the soils Fatick beneath canopy and Nguessine1 outside canopy.

Group II consists of soils outside canopy from Nguessine 2, Djilass and Fatick. They are characterized by heterogeneity of soils with high salinity and various pH rates. It means that salinity is the main factor that has influenced fungal communities structure, secondary supported by pH and perhaps others factors.

Diversity and richness of AMF morphotypes in *V. nilotica* rhizosphere

AMF spores in salt affected soils was estimated analyzing the number per 100 g of soil and identified morphotypes. Spores extraction by the wet sieving technique yielded mature spores in varying numbers depending on the soil sample origin (Figure 4). These spores are also varying in sizes and colors (yellowish, brown, black and white).

Spores richness is different according to soil origin and there was a high density on soils collected under cover compared to those from outside the canopy. This could be explained by the best development of AMFs in the presence of the host plant roots in favorable conditions

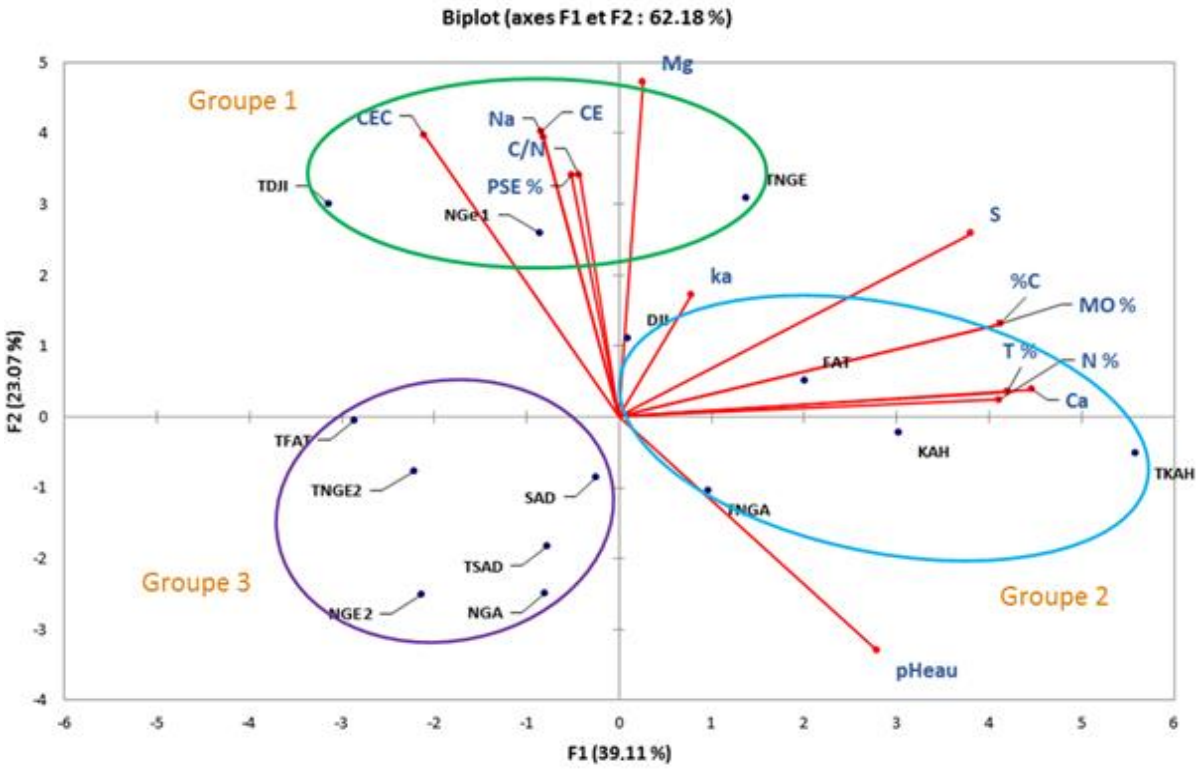


Figure 2. Comparative analysis of physio-chemical properties of soils from Ngane (NGA), Djilass (DJI), Sadioga (SAD), Fatick (FAT), Kahone (KAH), Nguessine (NGE) collected beneath canopy and outside canopy (T).

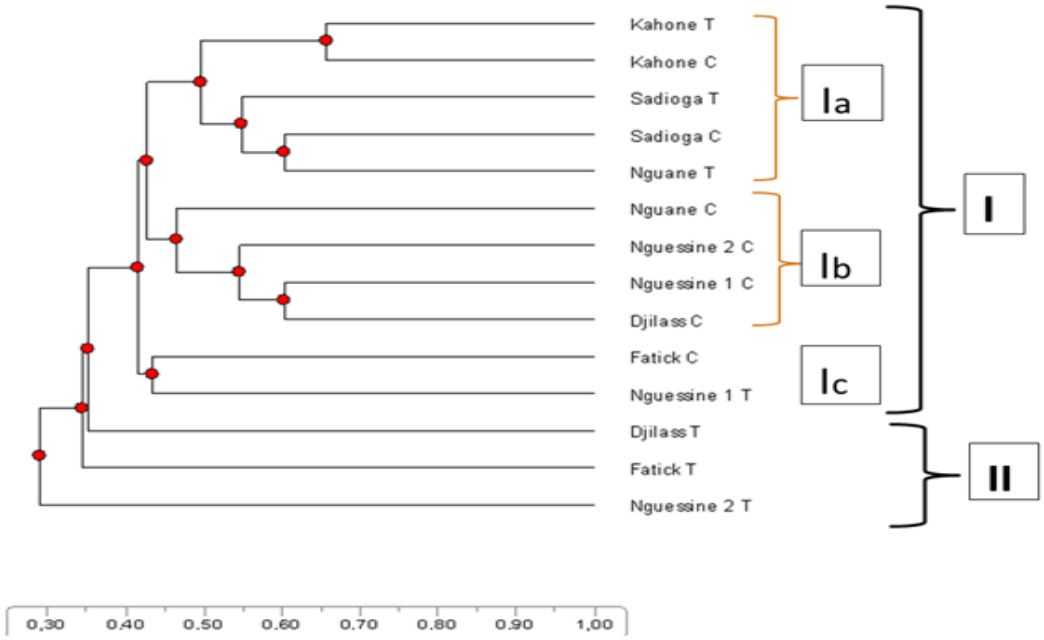


Figure 3. Dendrogram of similarity based on comparaison of the DGGE profiles of the ITS region of the 18S rDNA gene of the total soil fungal community, carried out with the Phoretix 1D tutorial version 10 software (Total Lab Ltd). Site name with C = beneath the canopy and with T = outside canopy.

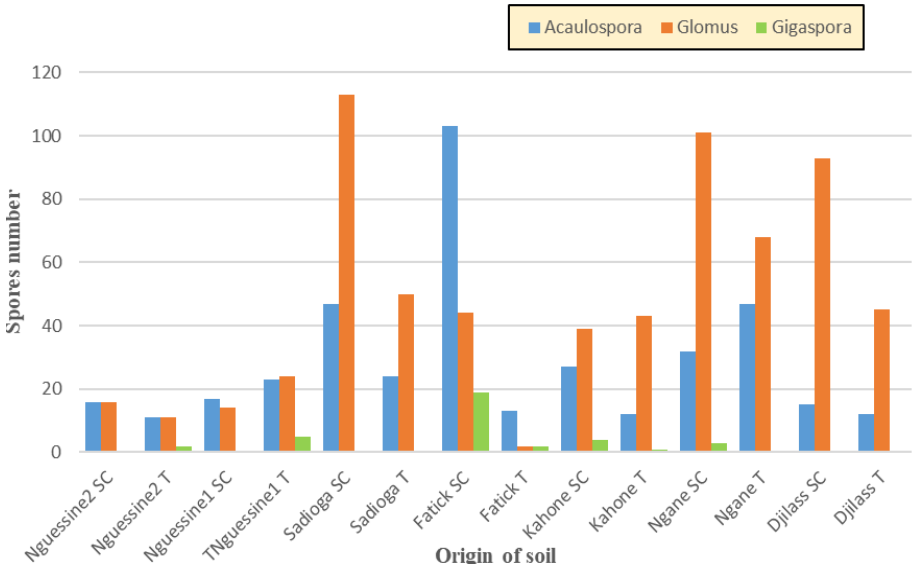


Figure 4. Numbers of AMF spores for each morphotype isolated per 100 g of rhizospheric soils beneath (SC) and outside the canopy (T) of *V. nilotica* in dry season.

and spores release nearly. Generally, the high salinities soils showed the highest number of spores indicating that spores are formed in adverse conditions.

Spores morphotypes identified in this study in all the sites appeared to belong to three genera: *Glomus (G), Gigaspora (Gi), Acaulospora (A)*; *Glomus* and *Acaulospora* are widespread in almost all soils whereas the genus *Gigaspora* was found on some soils (Figure 4).

Several spores morphotypes could not be identified at species level. Among identified spores, three species are related to the genus *Glomus* (*G. sp.1, G. sp.2, G. sp.3*), five species to the genus of *Gigaspora* (*Gi. rosea*, *Gi. aff. albida*, Gi. sp.1, sp.2, sp.3) and five species of the genus *Acaulospora* (*A. aff. colombiana* and *A. sp1, A. sp.2, A. sp3 and A. sp.4)*. *Gi. rosea* was found in Fatick and Nguessine, *Gi. aff. albida* was found in Kahone and *A. aff. colombiana* in Djilass (Photo 2).

Analysis of spores collected according to season showed that in dry season, soils under the canopy from Fatick, Ngane, Djilass and Sadioga showed high number of spores while soils outside the canopy of Kahone, Djilass, Nguessine, Fatick showed low spores density due to absence of host plant. In wet season, soils outside the canopy from Fatick, Sadioga, Kahone and Nguessine had a high number of spores while soils under capony of Kahone, Nguessine and Djilass showed low spore numbers. It can be noted that Sadioga had high spore number in both season; this could be explained by their low salinity and herbaceous development.

Comparative analysis of spores number from dry and wet seasons (Figure 5) revealed that spores number is highest in dry season in most of the soils, because under favourable conditions (wet season) the spores germinate and colonize the plants.

Mycorrhizal colonization

Microscopic observation of the colored root fragments of the different treatments showed a lack of mycorrhizal structures in un-inoculated plants roots. Vesicles and hyphae were observed in the plants inoculated with rhizospheric soils samples, meaning that these rhizospheric soils were rich in AMF strains infectives to *V. nilotica.* Because of many hyphae and vesicles observed in the roots (Photo 1), *V. nilotica* is a very mycotrophic plant.

Mycorrhization frequency and intensity estimated for soils collected in dry and wet seasons are presented in Figures 6 and 7. The intensity of mycorrhization is variable depending on the type of soil, season (wet or dry) and position to tree (beneath or outside canopy). The less salty soils showed the highest frequency and intensity of mycorrhization compared to high salty soils. This could be explained by the negative effect of salt on the AMFs multiplication. It is also noted that rhizospheric soils usually have significantly the highest values compared to samples outside the canopy showing the positive effect of the presence of the tree in mycorrhization. Plants with soil samples from Fatick, Kahone, Djilass, Sadioga, Ngane under the canopy have significantly higher colonization intensities and frequencies compared to uninoculated plants. The greater frequency is observed for Fatick in dry season and Ngane SC and Nguessing SC dominated in wet season.

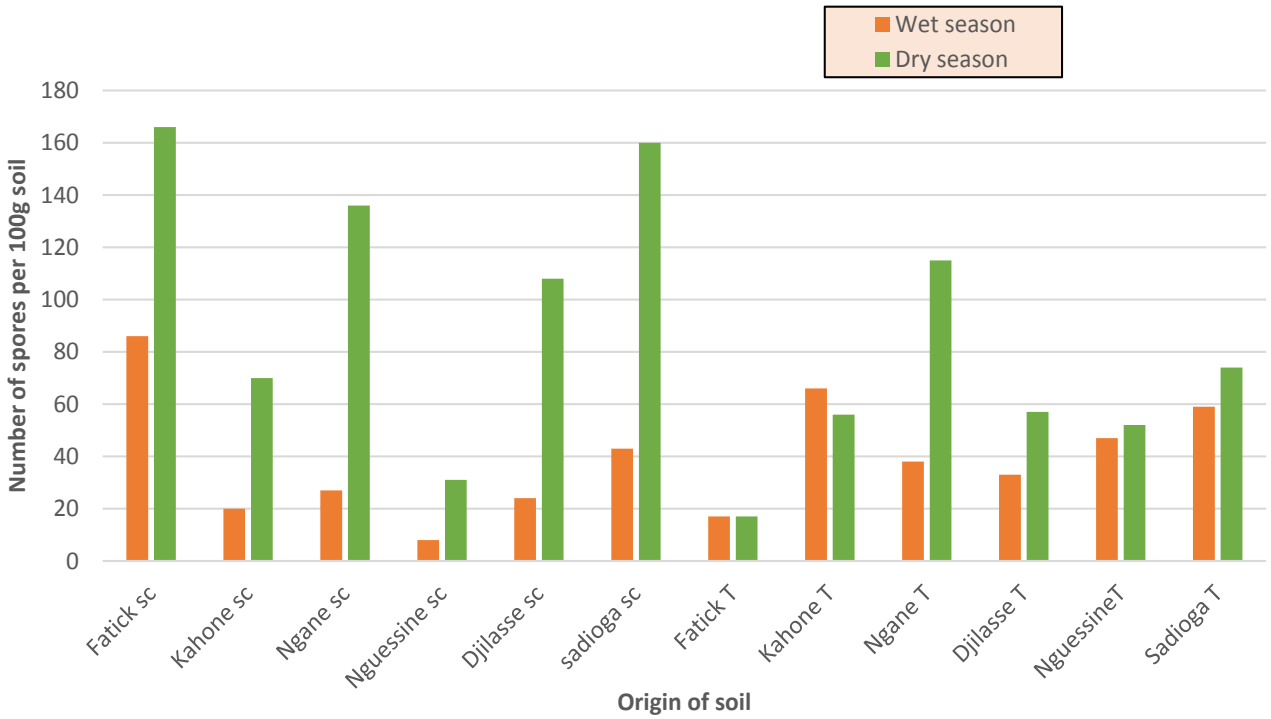


Figure 5.Spores number per 100 g of soil isolated from rhizospheric soils of *V. nilotica* from different sites, beneath (SC) and outside the canopy (T) in dry and wet season.

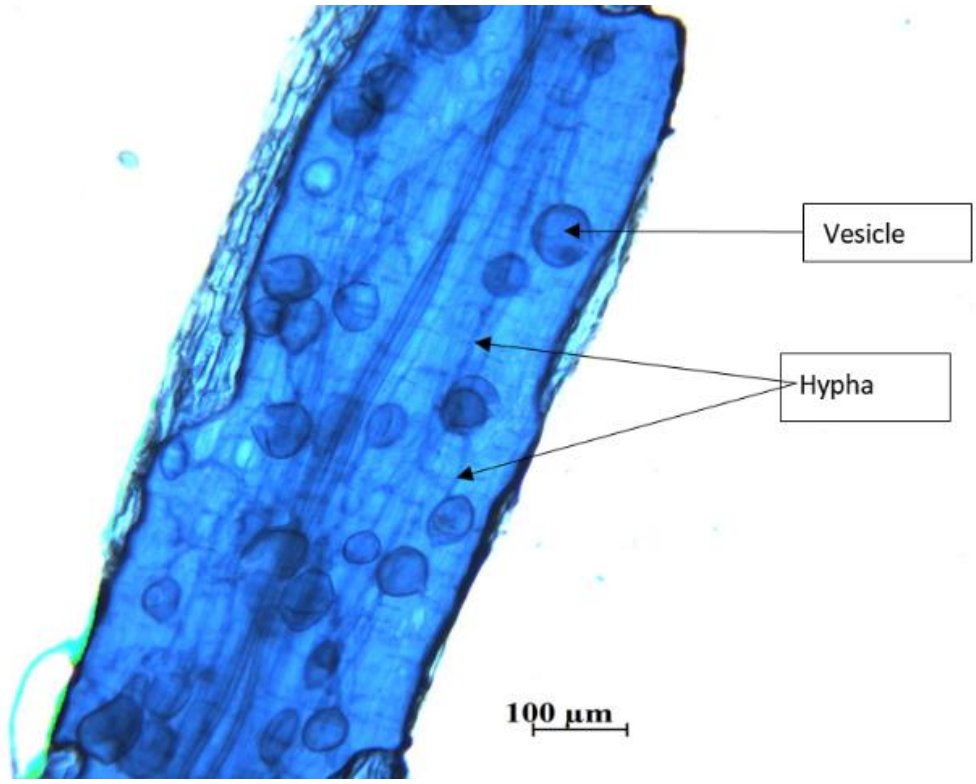
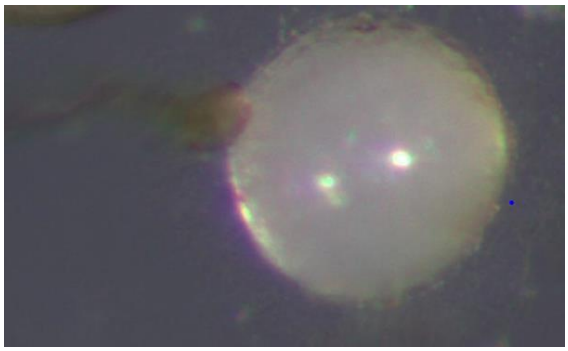
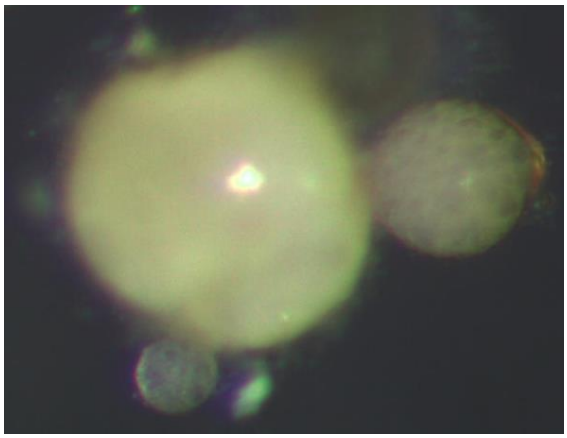


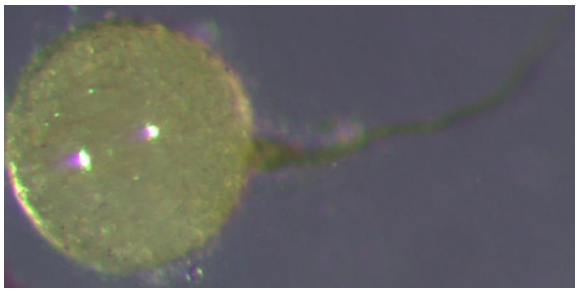
Photo 1. AMF Vesicles and hypha in *Vachellia nilotica* plant roots after trapping in greenhouse conditions.



***Gigaspora rosea* (Fatick)**



Acaulospora aff colombiana (Djilass)



Gigaspora aff albida (Kahone)

Photo 2. Spores morphotypes identified as known species in some soils.

Effect of mycorrhization on *V. nilotica* plant height and biomass

Our results showed mycorrhization have a positive effect on plant height (Figure 8), and soil samples collected beneath canopy lead to greater plant height compared to soils outside canopy. This effect varied according to soil origin and sampling period. In dry season (Figure 8A), soils beneath canopy from Nguessine2, Kahone, Fatick and NganeT showed a significant increase in the height of inoculated plants compared to plants control. DjilassT and Ngane SC soils showed no significant difference with plants control, because of their very low frequency and intensity of mycorrhization. In wet season (Figure 8B), soil beneath canopy from Nguessine, Fatick, Ngane, Djilass showed best results.

The impact of mycorrhization on plant total biomass is presented in Figure 9. Results showed a significant effect of inoculation on biomass of the inoculated plants which is greater than the control plants. However, increase of biomass varied according to the types of soil. Soils with high mycorrhizal frequencies and intensity are also those with high biomass ; this corroborated the positive effect of mycorrhization on plant biomass. The greater effect was

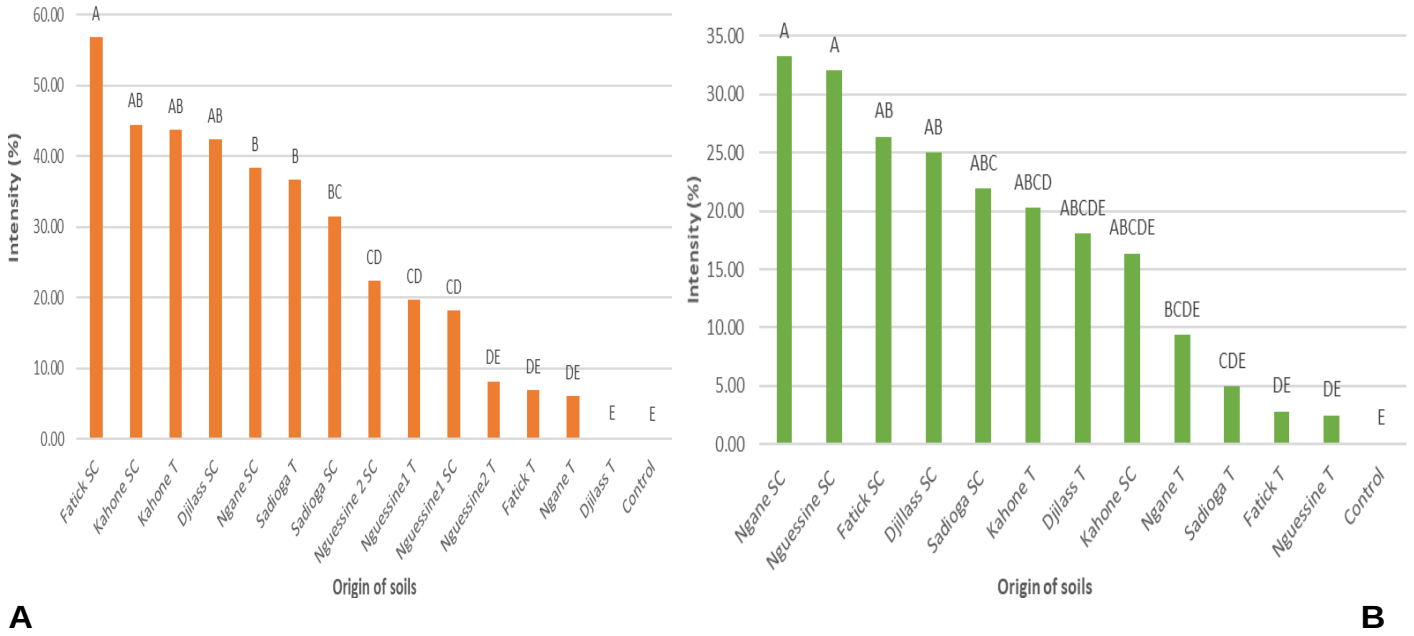


Figure 6. Mycorhizal intensities of *Vachellia nilotica* plants cultivated with rhizospheric soils samples collected beneath canopy (SC) and outside canopy (T) in dry (**A**) and wet (**B**) seasons. NB. Numbers with the same letter are not significantly different according to Newman et Keul's test. P< 0,0001.

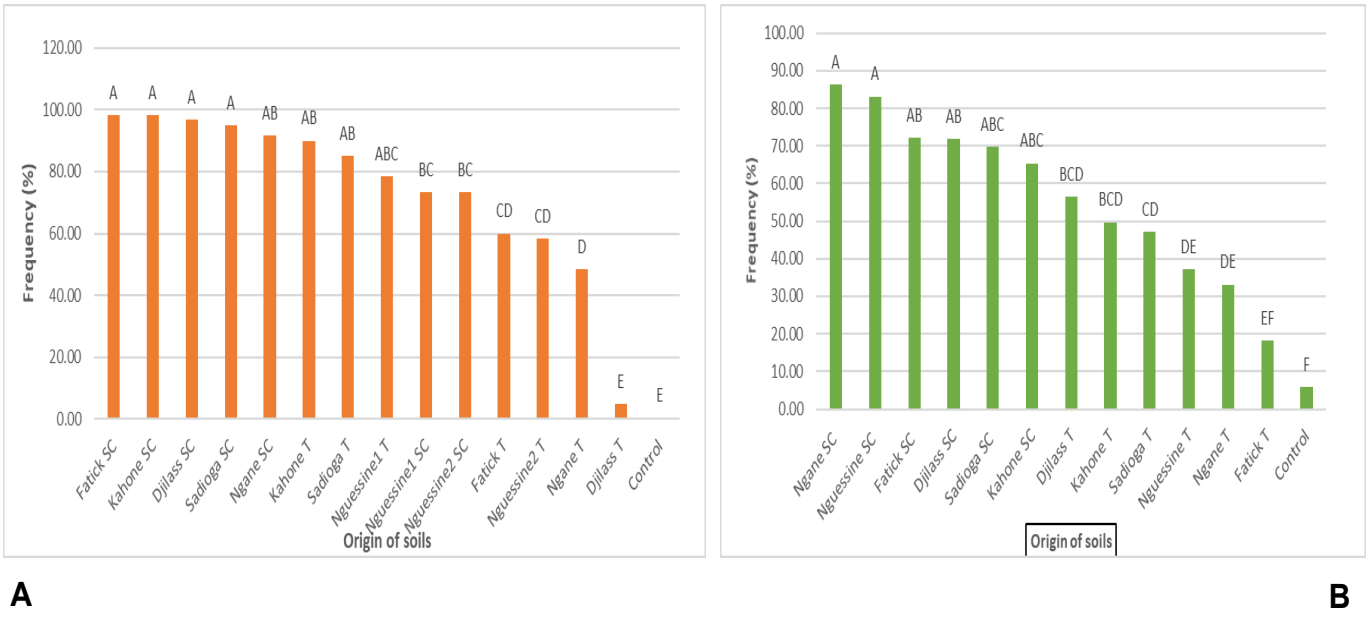


Figure 7. Mycorrhizal frequencies of *V. nilotica* plants cultivated with soils collected beneath canopy (SC) and outside canopy (T), in dry (A) and wet (B) seasons. NB. Numbers with the same letter are not significantly different according to Newman et Keul's test. P< 0.0001.

observed in dry season (9A) with soils from Sadioga, Kahone Fatick and Djilass and in wet season (9B) with those from Nguessine, Ngane, Kahone, Sadioga and Djilass, soils with the highest mycorrhizal colonization.

Otherwise, the positive impact of the mycorrhization on plant productivity (plant biomass and height) in all soils

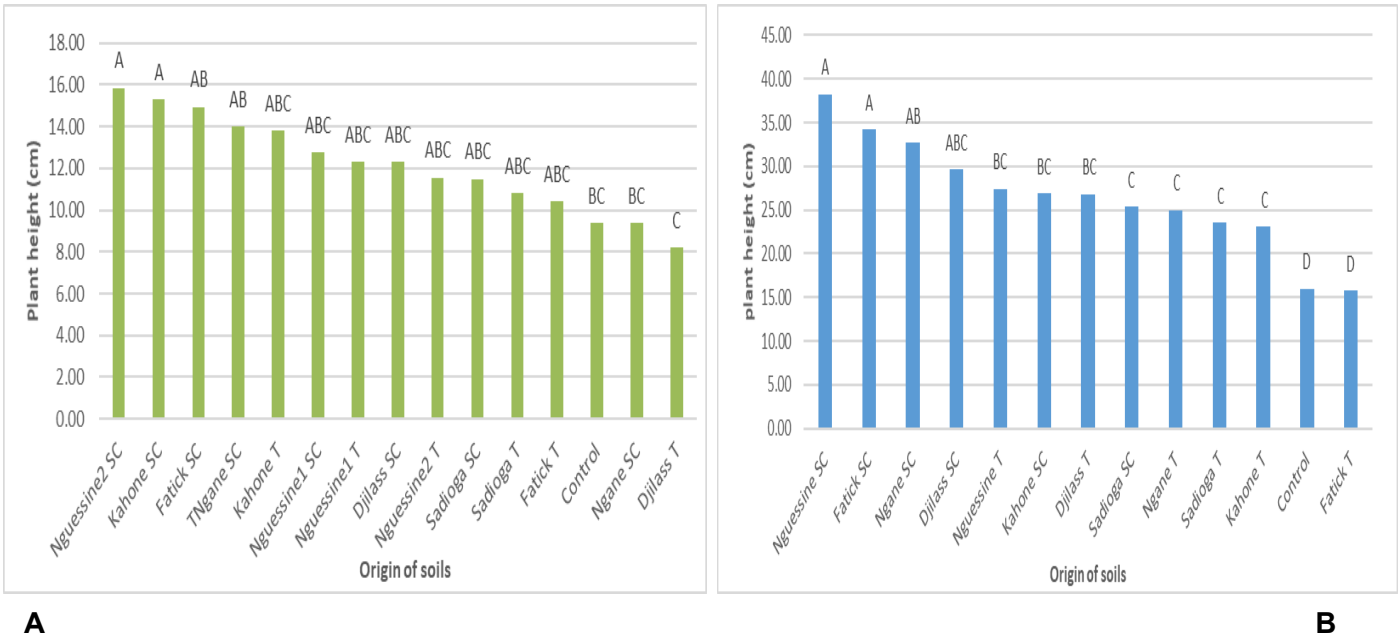


Figure 8. Effect of mycorhization on *V. nilotica* plant height cultivated in greenhouse with rhizospheric soil samples collected beneath canopy (SC) and outside canopy (T) in dry (A) and wet (B) seasons. Sites with the same letter do not differ significantly in plant biomass according to Newman Keul's test, P< 0.0001.

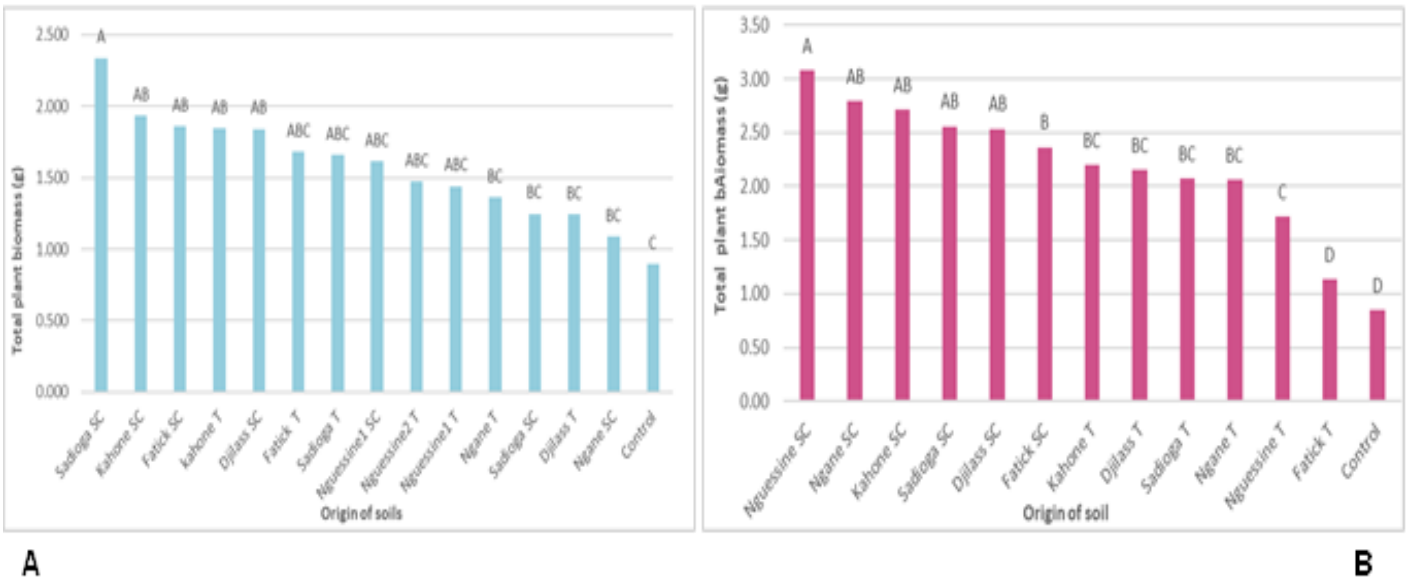


Figure 9. Biomass of *A. nilotica* plant cultivated with soils samples collected beneath canopy (SC) and outside canopy (T) in dry (A) and wet (B) seasons. Sites with the same letter do not differ significantly in plant biomass according to Duncan test, P < 0.0001.

especially in samples collected beneath canopy, as well in wet or dry season could mean that it has efficient AMF strains in rhizospheric soils of *V. nilotica* growing in salt affected areas.

DISCUSSION

Results show that AMF symbiotically associated with *V. nilotica* are present in the rhizosphere in salt affected

soils of Senegal as well as in dry and wet season. Our results revealed the presence of AMF viable spores related to the genus *Glomus* mainly, and to *Gigaspora* and *Acaulospora* in the *V. nilotica* tree rhizosphere grown in salt-affected areas.

Spores' numbers are more abundant in dry season compared to wet season. Silva-Flores et al. (2019) showed that there was a strong season effect of the soil in spores number at both sites, while physical-chemical parameters differed between sites.

In wet season, water availability would increase fungal mycelium growth for root colonization, leading to an increase of spore germination (Van Der Heijden et al., 1998; Ndoye et al., 2012). In dry season, soil salinity of most of our sites increases, but salinity has a negative effect on the development of AMFs (Bothe, 2012), hence the high number of spores was observed usually for the high salty soils. However, the effect is less marked beneath the tree canopy where the soil is influenced by the rhizosphere and where root exudates could mitigate the effect of salinity and allow a better development of AMF. Bargali and Bargali (2009) reported that *V. nilotica* improve soil fertility under its canopy because of increasing organic matter input, nutrient cycling through leaf litter and protection of soil from erosion. So, AMF diversity is not affected by salinity and only population size is affected as revealed in our results by the same AMF genus in all sites. Sene et al. (2012) reported the presence of *Glomus* only in rhizosphere of *V. nilotica* in non-saline soils. These AMF recovered are apparently widespread in many Senegalese soil because Sene et al. (2012), Ndoye et al. (2012) and Diop et al. (2015) described their association with other plant species. The presence of these AMF in salty soils could mean their adaptability in this harsh environment, as reported by many authors (Singh and Thomson, 1992; Giri et al., 2007; Bothe, 2012).

Only tree species were identified to known species: *G. rosea* was found in Fatick and Nguessine, *G. aff. albida* was found in Kahone and *A. aff. Colombiana* in Djilass and the others species were not affiliated to known species, so characterization will be continued to precise their taxonomical group.

Furthermore, soils factor such as salinity and pH appear to be decisive in structuring of AMF fungal communities as revealed in our results. Indeed, salinity has the main effect on diversity on total fungi, despite differences in soils properties. Analysis of total soil fungi targeted through the ITS region revealed that salinity decrease the total fungi diversity and soils with similar amount of salinity present similar profiles. In other words, salinity is the main factor that has influenced fungal community structure in salt affected soil. As revealed by Davidson et al. (2015), local environment conditions determine composition of AMF communities.

It appears that *V. nilotica* subsp *adansonii* can grow on different salt affected soils with different pH levels (acidic, basic) according to analysis of chemical properties of the rhizosphere soils. This corroborate the idea that *V. nilotica* tree is adapted to various edaphic conditions as reported by Giri et al. (2007) and by Chandrasekaran et al. (2014). Soils with a basic pH and low saline pH have the most important minerals (N, Ca, Mo, C, S, K, Mg) whereas acidic soils showed low mineral and organic matter contents.

Indigenous AMF in soils sampled in salt affected areas trapping using *V. nilotica* as trap plant indicated the infectivity of mycorrhizal strains present in rhizospheric soils as revealed by many propagules observed in plant roots. This corroborated the idea that *V. nilotica* is very mycotrophic and could be used as a trap culture plant for AMF associated with plants of the same genus, instead of the use of wheat as plant trapping.

Many authors such as Singh and Thomson (1992) and Giri et al. (2007) also reported that *V. nilotica* is a salinity-tolerant plant. However, mycorrhization is one of the factors contributing to this tolerance by improving hydromineral nutrition and soil nutrients release. This is also seen in our results that plants with high mycorrhizal colonization as observed for *V. nilotica* showed the highest plant biomass. Strains present in these salts affected soils might be efficient with *V. nilotica* and could therefore increase growth and biomass, corroborate the positive effect of AMF on plant development, especially in abiotic stress (Bothe, 2012). It should be noted that the soil of Kahone and Djilass present few nodules in the root system which could act in synergy with the mycorrhizae for the growth of the plant.

Conclusion

This work shows that rhizospheric soils of *V. nilotica* growing in salt affected areas have a great richness in indigenous AMF related to genus *Glomus, Gigaspora* and *Acaulospora*. Among AMF morphotypes identified, three are related to known species: *Gi. rosea*, *Gi. aff. albida* and *A. aff. Colombiana.*These strains can establish a good mycorrhization with *V. nilotica* and increase plant productivity (biomass and height), therefore could be used as inoculum for *V. nilotica* plants used to revegetalizise salt-affected soils. Also, AMF communities structure is correlated to soil salinity which has a negative effect on them. For further studies, it will be interesting to characterize genetically the real strains spp. that colonize plant roots and to determine the efficiency of each taxa separately on plant productivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

Gueye, O. (LCM/IRD-ISRA-UCAD, Dakar) and Gueye, O. S. (UCAD, Dakar) are thanked for their valuable technical assistance. This work was supported by PAPES funding program. The authors are grateful to the *Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation* (MESRI) of Senegal.

REFERENCES

Abbas G, Saqib M, Akhtar J, Basra SMA (2013). Salinity tolerance potential of two *Acacia* species at early seedling stage. Pakistan Journal of Agricultural Sciences 50(4):683-688.

Ashraf MY, Shirazi MU, Ashraf M, Sarwar G, Khan MA (2008). Utilization of salt-affected soils by growing some Acacia species. *In*: Khan MA, Weber DJ (eds) Ecophysiology of high salinity tolerant plants. Tasks for Vegetation Science (Springer, Dordrecht) 40:289-311.

Bâ AM, Sanon KB, Duponnois R, Dexheimer J (1999). Growth response of *Afzelia africana* Sm. Seedlings to ectomycorrhizal inoculation in a nutrient-deficient soil. Mycorrhiza 9:91-95.

Bâ AM, Dalpé Y, Guissou T (1996). Les Glomales d'*Acacia holosericea* et d'*Acacia mangium*. Bois et Forets des Tropiques 250:6-17.

Bargali K, Bargali SS (2009). *Acacia nilotica*: A multipurpose leguminous plant. Nature and Science 7(4):11-19.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in Plant Science 10:1068.

Bothe H (2012). Arbuscular mycorrhiza and salt tolerance of plants. Symbiosis 58:7-16.

Chandrasekaran M, Boughattas S, Hu S, Oh SH, Sa T (2014). A meta-analysis of arbuscular mycorrhizal effects on plants grown under salt stress. Mycorrhiza 24:611-625.

Cho NS, Kim DH, Eom AH, Lee JH, CHoi TH, Choi HY, Leonowicz A, Ohga S (2006). Identification of Symbiotic Arbuscular Mycorrhizal Fungi in Korea by Morphological and DNA Sequencing Features of Their Spores. Journal of the Faculty of Agriculture, Kyushu University 51(2):201-210.

Davidson J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, Johnson NC, Kane A, Koorem K, Kochar M, Ndiaye C, Pärtel M, Reier Ü, Saks Ü, Singh R, Vasar M, Zobel M (2015). Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349:970-973.

Diop I, Ndoye F, Kane A, Krasova-Wade T, Pontiroli A, Do Rego F, Noba K, Prin Y (2015). Arbuscular mycorrhizal fungi (AMF) communities associated with cowpea in two ecological site conditions in Senegal. African Journal of Microbiology Research 9(21):1409-1418.

Diouf D, Duponnois R, Ba AT, Neyra M, Lesueur D (2005). Symbiosis of *Acacia auriculiformis* and *Acacia mangium* with mycorrhizal fungi and *Bradyrhizobium* spp. improves salt tolerance in greenhouse conditions. Functional Plant Biology 32:1143-1152.

Dommergues Y, Duhoux E, Diem HG (1999). Les arbres fixateurs d'azote: caractéristiques fondamentales et rôle dans l'aménagement des écosystèmes méditerrannéens et tropicaux, avec référence particulière aux zones subhumides et arides. Editions ESPACES, Montpellier 34, 512 p. ISBN 2-907293-57-5.

Fall D, Bakhoum N, Fall F, Diouf F, Faye MN, Ndiaye C, Hocher V, Diouf D (2017). Improvement of tree growth in salt-affected soils under greenhouse conditions using a combination of peanut shells and microbial inoculation. Journal of Agricultural Biotechnology and Sustainable Development 9(5):34-44.

Gardes M, Bruns TD (1993). ITS primers with enhanced specificity for basidiomycetes-Application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Gardes M, Bruns TD (1996). ITS-RFLP matching for identification of fungi. In: Clapp JP. (Ed.). Methods In Molecular Biology. Species Diagnostics Protocols: PCR and Other Nucleic Acid Methods 416p. Humana Press Inc. Totowa, New Jersey 50:177-186.

Gerdemann JW, Nicolson TH (1963). Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46:235-244.

Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010). Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519-530.

Giri B, Kapoor R, Mukerji KG (2007). Improved Tolerance of *Acacia nilotica* to Salt Stress by Arbuscular Mycorrhiza, *Glomus fasciculatum* may be Partly Related to Elevated K/Na Ratios in Root and Shoot Tissues. Microbial Ecology 54(4):753-760.

Hanin M, Ebel C, Ngom M, Laplaze L, Masmoudi K (2016). New Insights on plant salt tolerance mechanisms and their potential use for breeding. Frontiers in Plant Science 7:1787.

LADA (2009). Land degradation assessment in Senegal (2009). Evaluation of soil degradation in Senegal», FAO project Report.

Van Der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396(6706):69-72.

Manga A, Ndiaye F, Diop TA (2017). Le champignon arbusculaire *Glomus aggregatum* améliore la nutrition minérale d'*Acacia seyal* soumis au stress salin progressif. International Journal of Biological and Chemical Sciences 11(5):2352-2365.

Muyzer G, de Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes encoding for 16S rRNA. Applied and Environmental Microbiology 59:695-700.

Muyzer G, Smalla K (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73:127-141.

Ndoye F, Kane A, Ngonkeu Mangaptché EL, Bakhoum N, Sanon A, Diouf D, Sy MO, Baudoin E, Noba K, Prin Y (2012). 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.) willd. International Scholarly Research Network Ecology13:1-13.

PAPIL (2013). Etude diagnostic et cartographie de la salinité des sols et des eaux dans les régions de Fatick et Kaolack. Final report.127p.

Philips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of British Mycological Society 55:158-161.

Raghuwanshi R, Upadhyay RS (2004). Performance of vesicular-arbuscular mycorrhizae in saline-alkali soil in relation to various amendments. World Journal of Microbiology and Biotechnology 20:1-5.

Sambou A, Ndour B, Cheng S, Senghor E (2010). Ligneous Species Tolerance in Acid Sulphated and Saline Soils of Sine Saloum: Case of Rural Community of Djilass and Loul Secene. Journal of Sustainable Development 3(2):174-186.

Sene G, Samba Mbaye R, Thiao M, Khasa D, Kane A, Manga A, Mbaye MS, Sylla SN (2012). The Abundance and Diversity of legume-nodulating rhizobia and arbuscular mycorrhizal fungal communities samples from deforested and man-made forest system in a semiarid sahel region in Senegal. European Journal of Soil Biology 52:30-40.

SES (2016). Ecomomic and social situation in Senegal. *ANSD Report*, 142p.

Singh S, Thomson FB (1992). Salt tolerance in some tropical tree species. Bois et Forêts des Tropiques 234(4):61-67.

Silva-Flores P, Bueno CG, Neira J, Palfner G (2019). Factors Affecting Arbuscular Mycorrhizal Fungi Spore Density in the Chilean Mediterranean-Type Ecosystem. Journal of Soil Science and Plant Nutrition 19:42-50.

Trouvelot A, Kouh J, Gianinazzi-pearson V (1986). Mesure du taux de mycorhization VA d'un système radiculaire: Recherche de méthodes d'estimation ayant une signification fonctionnelle. Les mycorhizes : Physiologie et génétique, 1er séminaire, 1-5 juillet 1985, INRA Dijon : 217-221.

White TJ, Bruns TD, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ eds. PCR Protocols: A Guide to Methods and Applications. Academic Press. New York pp. 315-322.

Zhu JK (2001). Plant soil tolerance. Trends in Plant Science 6:66-71.