



Research Article

# Diversity of Arbuscular mycorrhizal fungi associated to *Acacia seyal* (Delile) in semi-arid zone of Senegal

\*Anicet Manga<sup>1,4</sup>, Yolande Dalpé<sup>2</sup>, Abdoulaye Soumaré<sup>3</sup>, Tahir A. Diop<sup>4</sup>

<sup>1</sup>Section Productions Végétales et Agronomie, UFR des Sciences Agronomiques, de l'Aquaculture et des Technologies Alimentaires, Université Gaston Berger, BP 234, Saint Louis, Sénégal.

<sup>2</sup>Agriculture et agroalimentaire Canada, Centre de recherche et développement d'Ottawa 960 Carling Ave, Ottawa, ON K1A 0C6, Canada

<sup>3</sup>Laboratoire Commun de Microbiologie (LCM) IRD/ISRA/UCAD, Centre de Recherche de Bel Air, Dakar, Sénégal

<sup>4</sup>Laboratoire de Biotechnologies des Champignons, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.

*Acacia seyal* (Del.) is a multi-purpose leguminous tree growing in diverse habitats including saline areas and plays an important ecological role in semi-arid ecosystem in Senegal. In spite of that, the diversity of the arbuscular mycorrhizal fungi associated with this tree remains little known. In order to remediate to this lack of knowledge, studies were conducted at three locations characterized by differences in salt content to assess the arbuscular mycorrhizal fungi diversity. Rhizospheric soil samples and roots taken from adult *A. seyal* tree on each site were used to trap fungal cultures and isolated spores. This resulted in a morphological identification of the spores after five months of trap culture in greenhouse. A total of eight species of fungi were isolated, reflecting the low diversity of the species of arbuscular fungi associated with *A. seyal*. The isolated species belong to the family *Glomeraceae*, *Claroideo glomeraceae* and *Acaulosporaceae* and may represent the main species of arbuscular mycorrhizal fungi associated to the growth and development of *A. seyal* in a semi-arid environment.

**Keywords:** Morphological diversity, Glomeromycetes, Soil salinity

## INTRODUCTION

Drought and salinity constitute major environmental constraints which considerably limit plant production, especially in arid and semi-arid zone (Apse *et al.*, 1999). Under these hostile environments, plant species such as acacias (Family Fabaceae, subfamily Mimosoideae), are of major interest for soil remediation due to their adaptive capacity. Among *Acacia* species, *A. seyal* (Del.) a typical Sahelian tree, is a nitrogen-fixing species, belonging to one of over 60 African acacias. Native of the Senegal to Sudan Sahelian zone, *A. seyal* combines tolerance of periodically inundated heavy clay soils with major roles in fuel and fodder production in the southern edge of the Sahara desert (Hall, 1994). Tree, leaves and shoots provide forage, and wood is particularly used for charcoal. The branches are used for fencing and the fruits are often lopped by herders when forage decreases in dry season.

Talha gum is collected from the tree and a proportion is exported (Mohammed, 2011).

Because of its adaptive and symbiotic potential *A. seyal* plays an important ecological role in the Sahelian ecosystems. Its ability to associate with various microorganisms such as arbuscular mycorrhizal fungi (AMF) and rhizobia (Diouf *et al.*, 2010) contributes to its enhanced tolerance to environmental stresses while improving soil fertility.

**\*Corresponding Author:** Anicet Manga, Section Productions Végétales et Agronomie, UFR des Sciences Agronomiques, de l'Aquaculture et des Technologies Alimentaires, Université Gaston Berger, BP 234, Saint Louis, Sénégal. **E-mail:** anicet.manga@ugb.edu.sn **Tel.** +221 30 100 42 21, **Fax** +221 33 961 18 84

**Table 1:** Soils characteristics

	EC (mmho/cm)	pH	Absorbant complex Meq/100g				Total C (‰)	Total N (‰)	C/N	P <sub>2</sub> O <sub>2</sub> Olsen (ppm)	
			Ca	Mg	Na	K					
<b>Bambey</b>	0.49	5.5	1.60	0.60	0.09	0.04	2.39	0.238	10	11.5	Sandy
<b>Ngane</b>	28.30	5.6	5.70	3.96	12.4	0.47	2.54	0.238	11	13.7	Sandy
<b>Ndiafate</b>	100.80	3.6	27.0	15.6	29.1	0.13	6.14	0.308	20	16.0	Sandy
<b>Trapping soil</b>	3.55	8.5	8.06	0.63	0.41	0.06	Low	0.073	-	16.0	Sandy

EC: Electrical Conductivity, Ca : Calcium, Mg : Magnesium, Na : Sodium, K : Potassium, C: Carbone, N : Nitrogen, C/N : ratio Carbon on Nitrogen, P<sub>2</sub>O<sub>2</sub> : available Phosphorus

AMF are obligate biotrophic organisms associated with a wide range of vascular plants (Smith and Read, 2008). Their marked effects are often upon physiological aspects of host partner particularly under stress conditions as drought, and salinity (Porcel *et al.*, 2011; Augé *et al.*, 2015; Mo *et al.*, 2016; Evelin *et al.*, 2009; Soumaré *et al.*, 2015). AMF can promote plant growth (Ahanger *et al.*, 2014; Kim *et al.*, 2017) through improved plant nutrition and production of osmoregulators (Ruiz-Lozano et Azcon 2000; Laaziza *et al.*, 2003, Rabie, 2005; Zuccarini 2007; Ndiaye *et al.*, 2011). These symbiotic fungi utilize the carbohydrates that plants produce by photosynthesis (Wright *et al.*, 1998, Beligh *et al.*, 2014) and provide essential mineral and water inputs to plants.

A better knowledge of the taxonomic diversity of AMF and their specific functional relationships with plants is necessary for proper use of these terrestrial microorganisms especially in semi-arid environments. Understanding the mechanisms that enable the plants to grow and develop under these unfavorable conditions is crucial (Aroca *et al.*, 2013, Babu and Reddy, 2013).

Previous studies on the molecular (Manga *et al.*, 2007) and morphological (Belaye *et al.*, 2013) diversity led to better know the diversity of AMF associated with *A. seyal*. This study was carried out in various semi-arid zones characterized by different salinity levels with the objective to assess the diversity of AMF associated with *A. seyal*. We hypothesized that the AMF species communities in symbiosis with *A. seyal* vary according soils abiotic factors. A better understanding of AMF associated with *A. seyal* together with adapted choice AMF inoculants inputs at reforestation would improve, in a short and medium-term plant hydromineral nutrition and consequently favor plant health and enhance *Acacia* resistance to drought and soil salinity stresses.

## MATERIAL AND METHODS

### Sites characteristics

The study was conducted in three Senegalese localities belonging to the sahelo-soudanian field between isohyet 500 and 700 mm and covers the « peanut basin », which is the main area of peanut cultivation. This zone is characterized by a wooded shrubby savanna with a strong

predominance of *Faidherbia albida*, *Borassus sp.*, *Adansonia digitata*, *Cordyla sp.*, *Sterculia sp.* and *Combretum sp.* (CSE, 2003) and *Acacia seyal* notably associated with clay soil (MEPN, 1997). The soils of these localities are characterized by a decreasing gradient of salinity between the localities of Ndiafate (14°04'N; 16°10'O), Ngane (14°11'N; 16°05'O) which are in salted zone and Bambey (14°42' N, 16°29' O) located in a non-salted zone.

In the site of Ndiafate located in salted zone, the severity of the saline stress is marked by many salt crystals in the soil where only some woody and herbaceous plants species survive. In this site, it is also noted the presence of *Tamarix* for the ligneous family and *Borreria* for the herbaceous ones. The non-saline soils of Bambey carry an abundant population of acacia. The cortège of herbaceous plants is dominated by *Andropogon sp.*, *Pennisetum sp* and *Zornia sp.* all AMF associated plants.

### Soil analysis

Soil chemical analysis (table 1) were carried out by Laboratoire des Moyens Analytiques (IRD, Dakar). Total carbon and nitrogen analysis was carried out by the combustion system Thermo-Finnigan Flash EA 1112 (Thermo-Finnigan, France). Available phosphorus was measured by the method of Dabin (1965). Soil electrical conductivity (EC) and pH was measured on a 1:5 soil/water suspension (Rayment and Higginson, 1992). Exchangeable cations were measured by emission spectroscopy.

### Plant and soil sampling

From each sampling site, soil surrounding the root system and root samples were collected in six *A. seyal* individual plants randomly selected. *A. seyal* roots are easily detectable by their brown-red colour. They were separated manually and isolated from the rhizospheric soil or directly sampled from the plant root system. In term of root fresh weight, around 1200 g of roots were collected per location. For each plant, five samples of rhizospheric soil (0-20 cm of depth) were taken between 0 to 10 m from the tree. Thirty rhizospheric soil samples containing *A. seyal* roots were collected from the rhizosphere of *A. seyal* species in each of the three sites. *A. seyal* root samples were than manually collected from the rhizospheric soil. Soil samples

**Table 2** : Soil and root morphotypes isolated from Ndiagate, Ngane and Bambey sites.

	Ndiagate		Ngane		Bambey	
	Rhizosphere	Roots	Rhizosphere	Roots	Rhizosphere	Roots
<i>Acaulospora lacunosa</i> (Morton)	–	–	+	–	–	–
<i>Acaulospora scrobiculata</i> (Trappe)	+	–	–	+	–	–
<i>Rhizoglyphus aggregatum</i> (Schenck & Sm.) Sieverd., Silva & Oehl) Walker	–	+	–	–	+	+
<i>Septoglyphus constrictum</i> (Trappe, Walker & Schüßler) Sieverd., Silva & Oehl	–	–	–	–	–	+
<i>Claroideoglyphus etunicatum</i> (Becker & Gerd.) Walker & Schüßler	–	+	–	–	–	–
<i>Glomus lacteum</i> (Rose & Trappe)	–	–	–	–	–	+
<i>Rhizoglyphus microaggregatum</i> (Koske, Gemma & Olexia) Sieverd., G.A. Silva & Oehl	+	–	–	–	–	–
<i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck	+	–	–	–	–	–

+ : presence - : absence

from each site, were mixed together and stored in polythene bags at room temperature before use. Similar treatment was done with root samples.

### AMF Trap cultures

Two distinct types of inoculum were used to trap indigenous spores collected from harvesting sites: 1- rhizospheric soil containing a mixture of spores and fragments of mycorrhizas, and 2- *A. seyal* root fragments. A portion of harvested roots were previously stained in trypan blue according to the method of Phillips and Hayman, (1970) in order to check the presence of mycorrhizal colonization. To establish pot trap cultures, 500 g of inoculum was mixed first with autoclaved sand and used as substrate for the five pot culture replicates. Each pot included the equivalent 100 g of inoculum. Pots were seeded with maize and *A. seyal* as host plants. Five replicates of soil and five replicates of roots were recovered per harvesting sites for a total of 30 samples. Each sample was used as inoculant on maize and on *A. seyal* pot-cultures for a total of 60 pots. Pots were regularly watered at the field capacity and bi-monthly fertilized with a Long Ashton solution (Hewitt, 1966), for a five months period in the greenhouse of IRD/ISRA/UCAD research center, Bel Air (14°44'N, 17°30'O). Plants were maintained at temperature and daylight (average temperature day/night of 28°C, relative humidity of 75%, and photoperiod of about 14h).

### Morphological characterisation

#### Spores extraction and identification

After harvest, AMF spores were extracted from the substrate by wet-sieving and decanting technique according to the method of Gerdemann and Nicolson, (1963).

AMF spores were isolated and examined under a stereomicroscope (AmScope SE306-PZ-P), and grouped according to their morphotypes. Spores of each

morphotype were mounted in polyvinylalcohol–lactic acid–glycerol (PVLG) (Omar *et al.*, 1979) and PVLG mixed with Melzer's reagent (1 : 1, v : v). The taxonomic identification of spores to species and genus level was based on spore morphological characteristics as size, colour, pigmentation, ornamentation, characteristics of their wall and by comparison with herbarium specimens of the Canadian National Mycological Herbarium (DAOM) Ottawa Canada, reference type-specimens, species material provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invam.caf.wvu.edu>), and species descriptions (Blaszkowski 2012).

### RESULTS

The stained field root samples of *A. seyal* collected in the salted zones showed typical AMF structures, mainly coenocytic intraradical and extraradical hypha, intraradical vesicles, and arbuscules confirming the mycorrhizal status of the species.

Greenhouse pot-culture trapping allowed to isolate a total of 8 AMF morphotypes from the three harvested sites. Microscopic spore observation revealed that six of the morphotypes belong to the glomoid spore species and two to the acaulosporoid ones (genus *Acaulospora*) (Table 2). The different morphotypes varied according to the sampling location and the type of inoculum used for greenhouse trapping.

Four different AMF morphotypes were isolated from the Ndiagate site with the highest salinity level (EC 100.8). Three spore morphotypes were extracted from soil trapping and a single one from root trapping. For the locality of Ngane (EC 28.3), two distinct morphotypes were isolated, one from the rhizosphere and one from the roots. From Bambey, 3 different morphotypes were isolated, including a morphotype common to both the rhizosphere and the roots and two distinct morphotypes present only in the roots.

Some relationship can be detected between the presence of a particular AMF species and the site salinity and soil parameters. The two *Acaulospora* species, *A. lacunosa* and *A. scrobiculata* were isolated from the saline sites only and the two-species occurred in the medium saline soil of Ngane. Interestingly *A. scrobiculata* was extracted from pots inoculated with *Acacia* roots, a confirmation of the functional mycorrhizae between the two organisms. *Rhizoglyphus aggregatum*, *Septoglyphus constrictum* and *Glomus lacteum* morphotypes were detected only in the non-saline Bambey site. *R. microaggregatum* and *G. rubiforme* only in Ndiagate.

Concerning the type of inoculum used for AMF trapping, most of morphotypes were isolated from either the rhizosphere or *A. seyal* roots. Only *R. aggregatum* was propagated and isolated from both roots and the rhizosphere.

Five of the eight species isolated from the roots and the rhizosphere of *A. seyal* trees were detected from the high saline Ndiagate site (Figure 1). *R. microaggregatum*, *G. rubiforme* and *Claroideoglyphus etunicatum* were found

only at the high level saline soil of Ndiagate. The pot-cultures inoculated with *Acacia* roots confirmed the on site AMF colonization of *A. seyal* with two of the glomoid spore species *R. aggregatum* and *C. etunicatum*.

Surprisingly, the low-saline soils and roots of Ngane revealed the only presence of acaulosporoid spore species of *A. lacunosa* from soil inoculated and *A. scrobiculata* from root inoculated pot-cultures. None of the six glomoid spore species were detected in any of the trap-cultures.

In the non-saline site of Bambey, only glomoid spore species were detected. *R. aggregatum* was the only species extracted from both soil and root inoculated pot-culture. The morphological identification was based on the regular presence of phenomenon of internal spore proliferation described by Koske (1985) and confirmed by Blaszkowski (2012). Interestingly the two other AMF species detected, *S. constrictum* and *G. lacteum* were obtained from root inoculated pots. No *Acaulospora* spores were found in non-saline Bambey site.



**Figure 1:** Morpho-anatomical aspects of spores isolated from Ndiagate (Nd), Ngane (Ng) and Bambey (Ba) with *Glomus rubiforme* (A); *Claroideoglyphus etunicatum* (B); *Acaulospora scrobiculata* (C); *Acaulospora lacunosa* (D), *Rhizoglyphus aggregatum* (E); *Septoglyphus constrictum* (F).

## DISCUSSION

Many studies have been conducted in different ecosystems to better understand the taxonomic diversity of AMF associated with various plants (Bouamri *et al.*, 2006 ; Ndoye *et al.*, 2012 ; Sène *et al.*, 2012 ; Diop *et al.*, 2013 ; Rodríguez-Morelos *et al.*, 2014; Songachan *et al.*, 2015). Such studies aim to exploit the natural soil organisms diversity to improve survival and growth of plants. Knowledge of the soil mycorrhizal community followed by the strain isolation and greenhouse propagation would provide effective inoculants adapted to the arid and saline plant growth conditions (Wubet *et al.*, 2003; Sharmah and Jha, 2011; Suresh and Nelson, 2015). The majority of the plants of the arid and semi-arid regions live in symbiotic partnerships with different AMF (Brundrett, 1991; Juniper and Abbott, 1993; Diop *et al.*, 1994; Dalpé *et al.*, 2000; Silva *et al.*, 2014; Diop *et al.*, 2015) as confirmed with the present study for *A. seyal*.

The study sites were characterized mainly by their zero, moderate or high level of salinity. The diversity found in soil and in *A. seyal* roots seems to not be influenced by the soil salinity or the soil pH. Indeed, the soil of Ndiagate which had the lowest pH (3.6) and the highest NaCl content seemed to show a more important diversity. On the other hand, the soil of Ngane with a pH of 5.6 and a moderated content of Na showed a very low diversity made essentially of *Acaulospora* species.

Compared to AMF soil diversity found in the rhizosphere of other arid and semi-arid saline area, the *A. seyal* rhizosphere diversity observed in the three harvesting sites seems to be underestimated. Beauchamp *et al.*, (2006) found for example 30 AMF morphospecies in *Populus* and *Salix* rhizospheres in Arizona. A unique cycle of pot-culture trapping has been recognized to not be sufficient to induce the whole AMF community species to sporulate and may constitute a limiting factor in the expression of AMF diversity. One of the factors which limit the direct study of spore characteristics of the soil is the low level of the spores which can be collected (Lee *et al.*, 2013). Certain spores collected directly from the soil can be difficult to characterize because their walls undergo alterations because of attack by soil microorganism. Preliminary trapping could allow at the same time a multiplication of the spores, easier morphological identification and an improvement to the estimation of the species composition in an ecosystem. However, the conditions which prevail during the trap culture differ from those found in natural environment. These changes would be at the origin of the dormancy or the non-development of some AMF species when pot-cultured. The dominance of some AMF in culture could also be related to the sporulation and fast colonizing capacity of some species compared to others. The AMF who sporulate quickly in pot culture could colonize the roots of the plants in a more aggressive way, and/or adapt in a faster way to the change of the conditions of the soil than other species.

The impact of salinity on the AMF community can also play a significant role on spore germination, root colonization and AMF community structure (Yamato *et al.*, 2008; Juniper and Abbott, 2006; Campanelli *et al.*, 2013). The present study showed that arbuscular fungi associated with *A. seyal* varies according to the localities and remains low in term of trapped species diversity. The spores identified in the rhizosphere of the studied sites belong to 3 families according to the classification of Redecker *et al.* (2013): *Glomeraceae*, *Claroideoglomeraceae*, and *Acaulosporaceae* with a prevalence of species of the former family. These results, which indicate the predominance of *Glomeraceae* and/or *Acaulosporaceae*, have been reported in several studies carried out in various tropical, prairies, grassland or semi-arid habitats (Ndoye *et al.*, 2012 ; Sène *et al.*, 2012; Dai *et al.*, 2013; Diop *et al.*, 2013 ; Cai *et al.*, 2014 ; Silva *et al.*, 2014). However, the AMF *Glomeraceae* dominated in Ndiagate and Bambey sites while at Ngane site only *Acaulosporaceae* were detected. On the other hand, no species belonging to *Gigasporaceae* and *Scutellosporaceae* was found in the samples of the three localities after trap culture. The absence of *Gigasporaceae* and *Paraglomaceae* were previously observed by Wubet and al. (2003) on *Prunus africana* in tropical zone. These results are in conformity with those of Stutz and al. (2000) and of Muthukumar and Udaiyan (2002) whose work on arid and semi-arid regions revealed predominance of *Glomeraceae* and *Acaulosporaceae* species after pot-culture trapping. The observed site species distribution revealed that the abundance of species is not necessarily aligned to environmental parameters prevailing on the respective sites, soil factors may play a determining role in the AMF diversity as showed by Oehl *et al.* (2017).

The majority of the species described in this study seem typical from arid and semi-arid regions. The species *R. aggregatum* has been previously found associated with another leguminous tree, *Faidherbia albida* in Senegal in sahelian and soudano-Guinean zone by Dalpé *et al.* (2000) where a low AMF diversity had also been described in the soils of four localities of Senegal. These authors also found species like *R. microaggregatum* and *R. intraradices* which seem to proliferate in the arid and semi-arid regions (Estrada *et al.*, 2013). *R. aggregatum* and *R. microaggregatum* was also found by Shi *et al.*, (2007) in China respectively as the most frequent and the most abundant species. These two species may adapt their process to environmental stress conditions (Jacobson, 1997; Shi *et al.*, 2007).

Muthukumar and Udaiyan, 2002 described in the south of India in arid and semi-arid regions the presence of *A. scrobiculata* and *R. aggregatum*. *S. constrictum* isolated from the rhizosphere of *A. seyal* had also been described in various other environments like the *Tamarix* rhizosphere in arid and semi-arid steppes (Bencherif *et al.*, 2016), salted areas (Bencherif *et al.*, 2015), cultivated and non-

cultivated soils (Oehl *et al.*, 2009; Blaszkowski, 2012) or salt March and sand dunes (Estrada *et al.*, 2013). *A. scrobiculata* species has been previously found mainly in non-saline indigenous environments and cultivated soils (Estrada *et al.*, 2013, Burgenjargal and Lee 2010). Its first distribution in saline soil areas indicated its capacity to survive to detrimental environmental conditions.

## CONCLUSION

This study allowed to provide more informations on the diversity of mycorrhizal fungi associated with *A. seyal*. This made it possible to demonstrate the low diversity of the AMF associated with this tree in the Senegalese semi-arid and saline environment. The majority of the species described in this study seem typical from arid and semi-arid regions.

However, this fungal community despite its low representativeness could play a large role in *A. seyal* tolerance to environmental constraints. Molecular tools could help to detect more AMF diversity in semi-arid soils and complement the morphological studies to better identify the isolated spores. It would be interesting to continue the studies of diversity and the isolation of AMF associated with *A. seyal* that could be used in reforestation programs in order to produce and develop inoculum from endogenous spores to improve the tolerance of *A. seyal* to the constraints of environment.

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