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

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*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, Claroideoglomeraceae 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 herdsmen 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.

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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 sahelian-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, Cordyly 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 Ndiaeate (14°04'N; 16°10'O), Ngane (14°11'N; 16°05'O) which are in salted zone and Bamby (14°42' N, 16°29' O) located in a non-salted zone.

In the site of Ndiaeate 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 Bamby carry an abundant population of acacia. The cortege of herbaceous plants is dominated by Andropogon sp, Pennicetum 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 Higgison, 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 then manually collected from the rhizospheric soil. Soil samples

**Table 1:** Soils characteristics

<table>
<thead>
<tr>
<th></th>
<th>EC (mmho/cm)</th>
<th>pH</th>
<th>Absorptant complex (µg/100g)</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>C/N</th>
<th>P2O5 Olsen (ppm)</th>
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<tbody>
<tr>
<td>Ca</td>
<td>Mg</td>
<td>Na</td>
<td>K</td>
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<tr>
<td>Bambey</td>
<td>0.49</td>
<td>5.5</td>
<td>1.60</td>
<td>0.60</td>
<td>0.09</td>
<td>0.04</td>
<td>2.39</td>
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<tr>
<td>Ngane</td>
<td>28.30</td>
<td>5.6</td>
<td>5.70</td>
<td>3.96</td>
<td>12.4</td>
<td>0.47</td>
<td>2.54</td>
</tr>
<tr>
<td>Ndiaeate</td>
<td>100.80</td>
<td>3.6</td>
<td>27.0</td>
<td>15.6</td>
<td>29.1</td>
<td>0.13</td>
<td>6.14</td>
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<tr>
<td>Trapping soil</td>
<td>3.55</td>
<td>8.5</td>
<td>8.06</td>
<td>0.63</td>
<td>0.41</td>
<td>0.06</td>
<td>Low</td>
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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 Ndifate 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 Bamby, 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.

### Table 2: Soil and root morphotypes isolated from Ndifate, Ngane and Bambey sites.

<table>
<thead>
<tr>
<th></th>
<th>Ndifate</th>
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<th>Ngane</th>
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<th>Bamby</th>
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<tr>
<td></td>
<td>Rhizosphere</td>
<td>Roots</td>
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<td>Rhizosphere</td>
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<tr>
<td>Acaulospora lacunosa (Morton)</td>
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<tr>
<td>Acaulospora scrobiculata (Trappe)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Rhizoglosum aggregatum (Schenck &amp; Sm.)</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Septoglosum constrictum (Trappe, Walker &amp; Schüßler)</td>
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<tr>
<td>Ciarooideoglosum etunicatum (Becker &amp; Gerd.)</td>
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<td>Glomus lacteum (Rose &amp; Trappe)</td>
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<tr>
<td>Rhizoglosum microaggregatum (Koske, Gemma &amp; Olexia)</td>
<td>+</td>
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<tr>
<td>Glomus rubiforme (Gerd. &amp; Trappe)</td>
<td>Almeida &amp; Schenck</td>
<td>+</td>
<td>-</td>
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+ : presence  - : absence
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. *Rhizoglomus aggregatum*, *Septoglomus constrictum* and *G. lacteum* morphotypes were detected only in the non-saline Bambey site. *R. microaggregatum* and *G. rubiforme* only in Ndiate. 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 Ndiate site (Figure 1). *R. microaggregatum*, *G. rubiforme* and *Claroideoglomus etunicatum* were found only at the high level saline soil of Ndiate. 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 Ndiate (Nd), Ngane (Ng) and Bambey (Ba) with *Glomus rubiforme* (A); *Claroideoglomus etunicatum* (B); *Acaulospora scrobiculata* (C); *Acaulospora lacunosa* (D), *Rhizoglomus aggregatum* (E); *Septoglomus 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; Rodriguez-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 Abott, 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 Ndiafate 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 Ndiafate and Bambye 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 Udayian (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 Udayian, 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 arbuscular 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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