

# Tamarind (*Tamarindus indica* L.) parkland mycorrhizal potential within three agro-ecological zones of Senegal

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## Tamarind (*Tamarindus indica* L.) parkland mycorrhizal potential within three agro-ecological zones of Senegal.

**Abstract — Introduction.** Tamarind (*Tamarindus indica* L.) belongs to the Fabaceae family; it is a multipurpose tree with slow growth. In order to help improve its growth and development, we assessed mycorrhizal diversity of tamarind parklands in Senegal. **Materials and methods.** Three sites of tamarind populations were sampled for each agro-ecological zone in Senegal: the Sahelian zone (i), Sahelo-Sudan zone (ii) and Sudan zone (iii). Soil and root samples were collected in each site and used for arbuscular mycorrhizal (AM) spore isolation and root colonization assessment. We identified the mycorrhizal fungi from spore collections and evaluated the root mycorrhization rate, defined as percentage of roots colonized according to agro-ecological zones. **Results and discussion.** The results did not reveal a specific AM fungal strain associated with tamarind plants. Three arbuscular mycorrhizal fungi (AMF) were identified from spores on the genus level: *Glomus*, *Scutellospora* and *Acaulospora*. Tamarind sites with sandy soil texture (70–90%) and located in dry areas (Sahel and Sudano-Sahel zones) were shown to be rich in mycorrhizal propagules. High densities of soil AM propagules evaluated with the Most Probable Number method (MPN) were found in Niokhoul (1100 propagules per 50 g of soil), Sakal (790 propagules per 50 g of soil) and Mbassis (780 propagules per 50 g of soil). However, higher mycorrhizal colonization (11%) was observed in the Sahel agro-ecological zone compared with the Sudano-Sahelian and Sahelian zones (3%) of Senegal. **Conclusion.** Our study explored natural AMF diversity as a starting point to develop inocula to be used in commercial nursery production of tamarinds.

**Senegal / *Tamarindus indica* / arbuscular mycorrhizae / symbiosis / fungal spores / biodiversity**

## Potentiel mycorrhizien des parcs à tamariniers (*Tamarindus indica* L.) dans trois zones agro-écologiques du Sénégal.

**Résumé — Introduction.** Le tamarinier (*Tamarindus indica* L.) appartient à la famille des fabacées ; c'est un arbre à multiples usages et à croissance lente. Afin d'aider à améliorer sa croissance et son développement, nous avons cherché à évaluer la diversité mycorrhizienne associée aux parcs à tamarinier au Sénégal. **Matériel et méthodes.** Trois sites de peuplement de tamariniers ont été échantillonnés pour chaque zone agro-écologique du Sénégal : la zone sahélienne (i), la zone sahélo-soudanaïenne (ii) et la zone soudanaïenne (iii). Des échantillons de sols et de racines ont été prélevés dans chaque site et utilisés pour isoler des spores de mycorrhizes à arbuscules (MA) et évaluer le niveau de colonisation des racines. Nous avons identifié les champignons mycorrhiziens à partir des spores collectées et évalué le taux de mycorrhization des racines défini comme le pourcentage de racines colonisées dans chaque zone agro-écologique. **Résultats et discussion.** Les résultats n'ont pas révélé de souches AM qui seraient spécifiquement associées aux plants de tamarinier. Trois genres de champignons mycorrhiziens arbusculaires (AMF) ont été identifiés à partir des spores collectées : *Glomus*, *Scutellospora* et *Acaulospora*. Les sites de tamariniers avec sols à texture sableuse (70–90 %) et situés en zones arides (sahel et zone soudano-sahélienne) se sont révélés riches en propagules mycorrhiziennes. Les fortes densités de propagules AM dans le sol évaluées avec la méthode du nombre le plus probable ont été trouvées à Niokhoul (1100 propagules pour 50 g de sol), Sakal (790 propagules pour 50 g de sol) et Mbassis (780 propagules pour 50 g de sol). Toutefois, la zone agro-écologique du Sahel a montré une plus forte colonisation mycorrhizienne (11 %) que celles des zones soudano-sahélienne et sahélienne (3 %) du Sénégal. **Conclusion.** Notre étude a exploré la diversité naturelle des champignons mycorrhiziens arbusculaires ; c'est un point de départ pour élaborer des inoculum aptes à être utilisés en pépinière commerciale de production de tamariniers.

**Sénégal / *Tamarindus indica* / mycorrhize à arbuscule / symbiose / spore fongique / biodiversité**

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## 1. Introduction

In tropical ecosystems, wild fruit-bearing species play multiple roles in ecosystem biodiversity conservation and improvement of rural populations' food situation and income through sales and consumption of fruits. Among these species, we can cite *Ziziphus mauritiana*, *Balanites aegyptica*, *Tamarindus indica*, *Adansonia digitata* and *Ximenia americana*. Furthermore, wild fruit trees are used in traditional medicine and also as a source of wood [1].

Tamarind (*Tamarindus indica*), in particular, is a subsistence tree species, which is largely used for food. This tree species has a broad geographic distribution across the subtropics [2]. *Tamarindus indica* belongs to the Fabaceae family and is a multipurpose species: every part of the tree has some value [3, 4]. However, *T. indica* is one of the species affected by ecosystem degradation [5]. In the Sahel zone, tamarind trees are rarely planted, because they are slow-growing [6]. Elsewhere, tamarind has been documented to bear fruit as early as (4 or 5) years after planting.

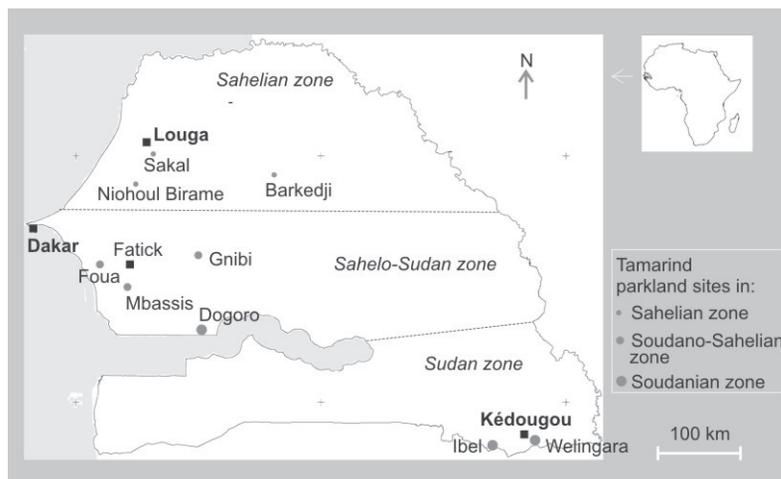
The diversity of arbuscular mycorrhizal (AM) fungi and their broad or narrow association with distinct plant species in natural environments are crucial information in the understanding of the ecological role of AM fungi in plant co-existence. This knowledge is also needed for appropriate mycorrhization of nursery-grown seedlings for reforestation efforts [7]. The current interest in

applying low-input in crop production agro-technology methods emphasizes the study and management of microbial interactions in soil-plant interfaces. Mycorrhizal associations have been shown to stimulate tree growth, and most tropical tree species are associated with arbuscular mycorrhizae that contribute to mineral nutrition and growth [5, 4]. One critical step for successfully applying arbuscular mycorrhizal fungus technology is the selection of effective fungal isolates on the plantation sites to be used as plant inoculants. For this purpose, it is recommended to test native ecotypes together with those considered to be "highly effective" from established culture collections [8]. Therefore, natural diversity of arbuscular mycorrhizal fungi in the root-associated soil from established tamarind plantations in the region should be analyzed. Very little is known on arbuscular mycorrhizal fungi that are associated with tropical fruit trees, including tamarind [4]. Previous work has, however, highlighted mycorrhizal dependency of tamarind [5]. The objectives of our study were (1) to characterize the mycorrhizal diversity of the rhizosphere associated with tamarind in situ in Senegal through three agro-ecological zones of Senegal, and (2) to evaluate the mycorrhizal tamarind parkland potential and tamarind root colonization rate.

## 2. Methodology

Three tamarind population sites per each of the three agro-ecological zones in Senegal [the Sahel zone (i), Sahelo-Sudan zone (ii) and Sudan zone (iii)] were sampled (figure 1). For each site, ten trees were randomly selected. Three soil samples (250–300 g) and fine tree roots (diameter less than 1 mm) were taken under each tree using a caliper gauge at (1, 3 and 5) m distance from the trunk, respectively, at a depth ranging from 0–40 cm. All samples from one site were mixed into one composite sample and labeled [6]. These soil samples were then air-dried in the laboratory and kept for one month at room temperature (4 °C). For each given site, part of the sample (200 g) was used for a physico-chemical analysis (IRD soil laboratory,

**Figure 1.** Tamarind (*Tamarindus indica* L.) arbuscular mycorrhizal fungi (AMF) collection sites within three agro-ecological zones of Senegal (Source: DTGC, administrative division of 2002, field data of 2007, production: ISRA / LERG, 2009).



Dakar, Senegal) and another part for arbuscular mycorrhizal fungus spore isolation.

### 2.1. Tree root colonization

The gridline method [9] was used to evaluate root colonization by arbuscular mycorrhizal fungi. Fine roots were selected and divided into test tubes. Roots were then stained with Trypan blue (0.05%) to highlight the characteristics of the arbuscular mycorrhizal infection structures. The root infection level was estimated using the gridline intersect method [9]. Histological observations were carried out under the microscope (40×) by depositing root fragments (0.5 cm) on a Petri dish under a squared-bottom grid line. Observations were made by following the horizontal and vertical lines on the Petri dish squared-bottom; each mycorrhization root point intersecting with a vertical or horizontal line was counted. The number of intersections of the mycorrhized root points was divided by the total number of intersections (in horizontal and vertical directions). The mean of this ratio for each line (vertical and horizontal) was summed. Mycorrhization frequency of the root sample was obtained by the following formula [9]:  $F = (N_{mh} + N_{mv}) / (N_{iv} + N_{ih})$ , where  $N_{mh}$  and  $N_{mv}$  are mycorrhized root intersections on horizontal and vertical lines, respectively, whereas  $N_{iv}$  and  $N_{ih}$  represent total number of root intersections with vertical and horizontal lines, respectively.

### 2.2. Viable spore propagules in parklands obtained from trap cultures

The density of viable spore propagules was evaluated by using the Most Propagule Number method: mycorrhizal soil infectivity values (MPN). Sterilized (120 °C for 2 h) soil substrate (sandy) and soil samples from each site were mixed at six levels of dilution. Each level of dilution was repeated five times in experimental buckets (of 0.5 L).

– Dilution  $10^{-1}$ : 30 g of non-sterile soil + 270 g of sterilized soil = 300 g (1). From this 300 g, 250 g was taken and distributed in five repetitions at a rate of 50 g per bucket; of the remaining 50 g, 30 g was taken for dilution  $10^{-2}$ ;

– Dilution  $10^{-2}$ : 30 g of dilution  $10^{-1}$  + 270 g of sterilized soil = 300 g;  
 – Dilution  $10^{-3}$ : 30 g dilution  $10^{-2}$  + 270 g of sterilized soil = 300 g;  
 – Dilution  $10^{-4}$ : 30 g dilution  $10^{-3}$  + 270 g of sterilized soil = 300 g;  
 – Dilution  $10^{-5}$ : 30 g dilution  $10^{-4}$  + 270 g of sterilized soil = 300 g;  
 – Dilution  $10^{-6}$ : 30 g dilution  $10^{-5}$  + 270 g of sterilized soil = 300 g.

*Zea mays* L. seeds were used as a trap crop and were first disinfected in bleach (3 min) then rinsed and pre-soaked in distilled water for 30 min. Seeds were kept in the dark to germinate in Petri dishes at 30 °C. After 3 days, seedlings were planted in pots and transferred to the greenhouse. Seedlings were watered daily to maintain soil at field capacity for 6 weeks. Fine roots (diameter less than 1 mm) were observed through a binocular magnifying glass (40×). Mycorrhization points (defined as a mycorrhization structure) of roots were counted after coloration by Trypan blue according to the law of the “whole or nothing” [9] (a plant is regarded as mycorrhized when mycorrhization roots are found and/or a figure of colonization is observed). The most probable number of propagules was estimated on roots following Cochran tables (1950).

### 2.3. Arbuscular mycorrhizal fungi spore diversity associated with *Tamarindus indica* L.

The natural diversity of AMF in the root-associated soil from long-term established tamarind trees was analyzed. Soil samples taken from tamarind parklands (figure 1) were used for AMF trapping in greenhouse conditions. Two distinct types of inocula were made up from each locality: (1) field soil without tamarind roots; and (2) fine tamarind roots obtained from the selected fields.

Tamarind and maize plants were used as mycorrhiza plant traps. Sterilized sea sand was used as (growing) substrate. The experiment lasted for 5 months in the greenhouse. After harvest, soil substrate and plant roots were collected and preserved in bags at 4 °C. Arbuscular mycorrhizal fungi spores were isolated by the humid extraction method [10–12]. Spores in the soil samples

**Table I.**

Tree (*Tamarind indica* L.) root colonization frequency assessed from soil samples collected in three sites for each of three agro-ecological zones in Senegal.

Sites	Root colonization rate (%)	Agro-ecological zones	Root colonization rate (%)
Niohoul	14.90 a	Sahelian	11.17 a
Barkedji	6.40 bc		
Sakal	12.21 ab		
Foua	4.04 c	Sudano-Sahelian	5.72 b
Gnibi	1.5 c		
Mbassis	11.63 ab		
Ibel	2.45 c	Sudanian	3.85 b
Welingara	5.12 bc		
Dogoro	3.98 c		

Means followed by the same letter in the same column are not significantly different according to the Student-Newman-Keuls test ( $p < 0.05$ ).

were extracted following the wet sieving method of Gerdemann and Nicholson [13]. For each soil sample, 200 g of soil were used for spore extraction. This viable spore extraction was worked out by the sucrose centrifugation technique of Daniel and Skipper [13]. Arbuscular mycorrhizal fungi spores were isolated by micropipette under a binocular magnifying glass (40×). Spores isolated were examined microscopically and identified on the genus level according to the taxonomic system proposed by Morton [14]. Original descriptions were consulted and spore morphology was compared with an internet-published reference culture database established by Morton<sup>1</sup>. Spores were also compared with freshly formed arbuscular mycorrhizal fungi spores from trap cultures originating from the same field site. Spores were observed using reflected-light illumination provided by fiber optics. The spores were mounted in water (for stereomicroscopy only). At least five spores of each arbuscular mycorrhizal fungi genus were mounted to observe their morphology. Only apparently viable spores were used for identification.

#### 2.4. Data analyses

The root colonization rate was obtained following formula (F) and data subjected to the

<sup>1</sup> see [http://invam.caf.wvu.edu/Myc\\_Info/Taxonomy/species.htm](http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm)

ANOVA test (Statistix 8.1). Mean values were separated with the Student–Newman–Keuls test. Arbuscular mycorrhizal fungus spore types and soil data were analyzed using multiple component analysis methods with the SAS V7 package [11]. To represent the complex multivariate relationships among variables, multiple component analysis (using Partial Least Squares regression: PLS) was performed on the correlation matrices and results were expressed as a diagram.

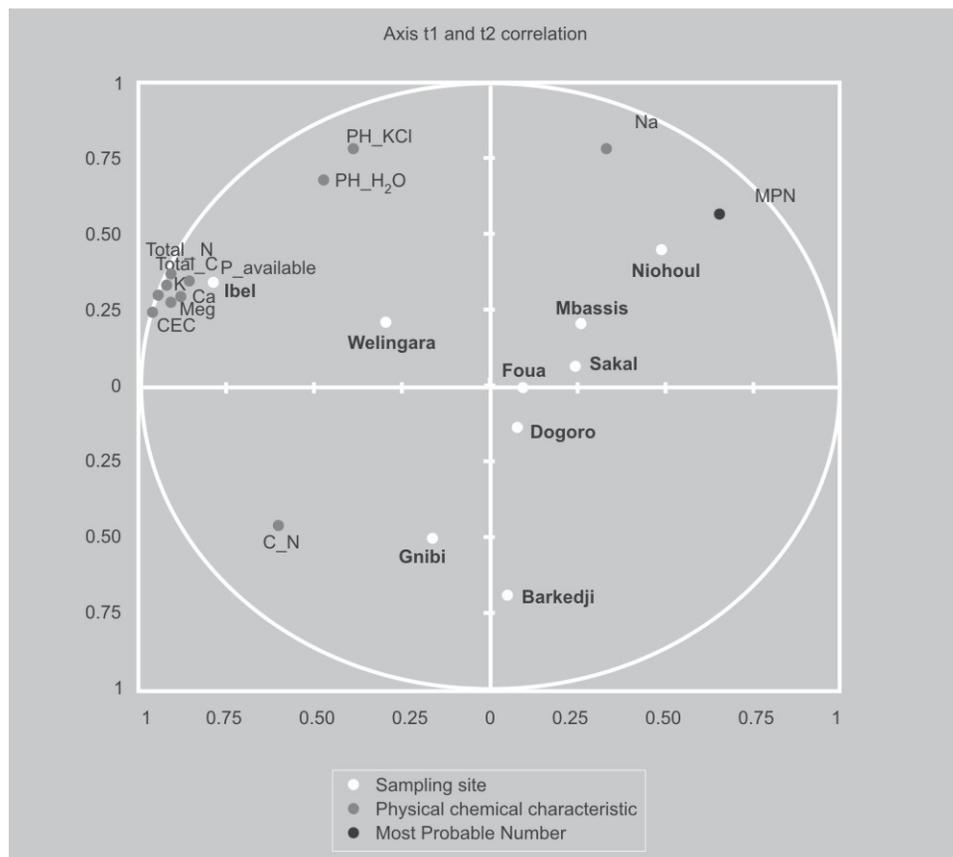
### 3. Results and discussion

#### 3.1. Root colonization

Tree root colonization rates per site and per agro-ecological zone were evaluated (table I). It appears that trees from the Sahelian zone are more colonized by mycorrhizae (11.17%) than in the Sudano-Sahel and Sudan zones. In addition, our results showed a decrease in soil fertility (4.60%) of soil organic matter in the Sudan zone compared with the Sahel (0.34%) (table II). These results corroborate those found on banana vitroplants in Cameroon [15]. Young banana vitroplants mycorrhized better (highest root colonization rate) on poor soil (low soil carbon level). This could be explained by an adaptation of plants towards different stresses suffered (water deficit, low soil fertility level) in arid and dry environments [16].

**Table II.** Physico-chemical properties of soil samples collected in three sites for each of three specific agro-ecological zones in Senegal.

Sites	Agro-ecological zone	pH <sub>H<sub>2</sub>O</sub>	N (total) %	Organic matter %	P available mg*kg <sup>-1</sup>	Soil texture (%)		
						Sand	Limon	Argile
Sakal	Sahelian	6.53	0.03	0.34	14	92	6.2	1.8
Niohoul		6.17	0.04	0.33	10	90.5	6.1	3.4
Barkedji		4.73	0.04	0.44	13	97.2	1.5	1.3
Foua	Sudano-Sahelian	6.21	0.06	0.66	21	86.7	3.7	9.6
Mbassis		6.28	0.05	0.56	7	82.3	9.9	7.8
Gnibi		5.85	0.08	0.98	45	96.4	1.5	2.1
Ibel	Sudan	6.94	0.41	4.60	248	68.3	21.4	10.3
Welingara		7.22	0.22	2.63	55	66.8	18.7	14.5
Dogoro		6.21	0.08	0.93	15	79.4	11.3	9.3

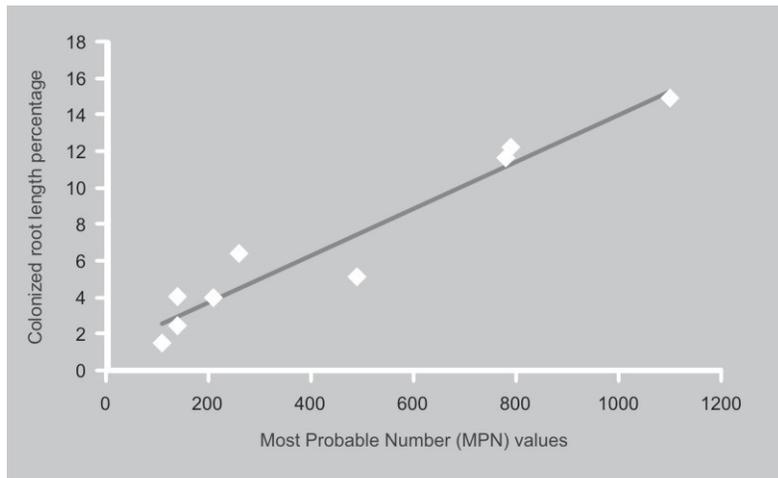


**Figure 2.** Tamarind parkland soil physico-chemical properties in relation to Most Probable Number (MPN), assessed from samples in three agro-ecological zones of Senegal.

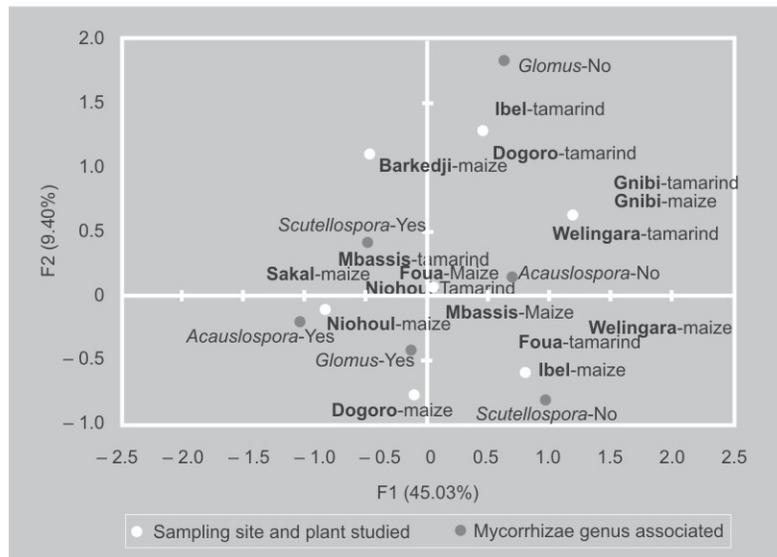
### 3.2. The Most Probable Number (MPN) found in trap cultures on *Zea mays*

Multiple component analysis on correlation matrices (using Partial Least Squares regres-

sion) indicated that the density of viable arbuscular mycorrhizal propagules (MPN), soil pH (water), pH (KCl) and sodium (Na) of the soil are closely related (figure 2). In this diagram, other soil factors, total carbon,



**Figure 3.** Relationship between the Most Probable Number (MPN) values and the colonized root length percentage for tamarind (*Tamarindus indica* L.) arbuscular mycorrhizal fungi (AMF) samples in Senegal.



**Figure 4.** Cluster analysis of mycorrhizal diversity associated with tamarinds studied in parklands of three agro-ecological zones of Senegal.

the carbon-nitrogen ratio (C/N), and available phosphorus, calcium, potassium and soil capacity exchange (CEC) are relatively distant from arbuscular mycorrhizal propagule number. Previous studies indicated that one of the factors known to influence spore numbers is soil pH. Nevertheless, the differences in pH (as well as in all other abiotic factors studied) observed between the

sites studied did not significantly impact arbuscular mycorrhizal propagule density. Differences in pH are also known to have an impact on spore viability but not on mycorrhizal infection [12]. As in previous research, our results do not show evidence for any soil pH influence on mycorrhization. But these results show a real soil pH influence on the soil arbuscular mycorrhizal propagule density [17]. However, the sandy soil texture (70–90%) of the Sahelian zone to Sudano-Sahel zone sites (Niohoul, Mbassis, Sakal) are also related to the MPN value. These results of MPN values follow those obtained on tamarind root length colonized percentage. The percentage of roots colonized increased with increasing MPN value (figure 3) ( $R^2 = 0.93$ ,  $p \leq 0.05$ ). However, in previous studies, it was shown that the percentage of root length colonized and the MPN values were affected by plant species and soil properties; in particular, soil texture and soil pH [18].

### 3.3. Mycorrhizal diversity

Three genera of arbuscular mycorrhizal fungi (*Acaulospora*, *Glomus* and *Scutellospora*) were associated with the tree under study. These genera, described by various authors [14, 19, 20], were observed in all tamarind parklands across Senegal and whatever the trap culture used (tamarind and maize). However, multiple component factorial analysis indicates that these mycorrhizal genera are related to sites (Sakal, Niohoul, and Mbassis) characterized by sandy soils (70–90%) (figure 4). *Glomus* spp. and *Scutellospora* spp. are related to axis 1 (45.03%), and axis 2 (9.40%) is covered by *Acaulospora* spp. These results indicate that *Tamarindus indica* L. has broad-spectrum mycorrhizal association; this was also found by previous studies [4, 5, 21]. Our results were unable to identify specific arbuscular mycorrhizal fungi associated with tamarind because we only worked on the genus level (figure 4). Similar results were obtained by various authors [3, 4]; they identified 13 arbuscular mycorrhizal fungi species associated with tamarind specifically in arid areas in order to improve water and mineral nutrition.

However, in dry areas of Africa, the best known are the *Glomus* and *Gigaspora* genera [10, 22]. *Glomus* and *Acaulospora* spores are significantly more numerous ( $P < 0.05$ ) than *Scutellospora* spores in all studied tamarind parklands. This was found by some previous research [23–25]. It was reported that *Glomus* is considered to be the most abundant of all arbuscular mycorrhizal fungi. *Glomus* is an obligate symbiotic fungus and not very host-specific. It was found that *Acaulospora* occurs with a wide range of soil types and host species [26]. *Scutellospora* were relatively low in number, in agreement with findings of various authors who found that *Scutellospora* spp. were exclusively found in farmed soils [27]. These results are contrary to those found by other authors [10, 12] which indicated that *Acaulospora* spp. and *Scutellospora* spp. tended to be more numerous than *Glomus* spp. on non-tilled soil such as tamarind parkland. Soil management may influence diversity and the root mycorrhization rate [28, 29]. This study emphasizes the importance of exploring and exploiting the natural diversity of arbuscular mycorrhizal fungi as a starting point to formulate inoculants as bio-fertilizers able to improve growth and productivity of parkland Tamarind.

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### **Potencial micorrícico de parques de tamarindo (*Tamarindus indica* L.) en tres zonas agroecológicas del Senegal.**

**Resumen — Introducción.** El tamarindo (*Tamarindus indica* L.) pertenece a la familia de las fabáceas; es un árbol cuyos usos son múltiples y cuyo crecimiento es lento. Con el fin de mejorar su crecimiento y su desarrollo, procuramos evaluar la diversidad micorrícica asociada a los parques de tamarindo en Senegal. **Material y métodos.** Se sacaron muestras de tres emplazamientos de rodales de tamarindos para cada zona agroecológica del Senegal: la zona saheliana (i), la zona sahelo-sudanesa (ii) y la zona sudanesa (iii). Se tomaron muestras de suelos y de raíces en cada emplazamiento, y se emplearon para aislar esporas de micorizas de arbusculas (MA) y para evaluar el nivel de colonización de las raíces. Identificamos los hongos micorrícicos a partir de esporas recogidas y evaluamos el nivel de micorrización de las raíces, definido como el porcentaje de raíces colonizadas en cada zona agroecológica. **Resultados y discusión.** Los resultados no mostraron cepas AM específicamente asociadas a las plántulas de tamarindo. Se identificaron tres tipos de hongos micorrícicos arbusculares (AMF) a partir de las esporas recogidas: *Glomus*, *Scutellospora* y *Acaulospora*. Los emplazamientos de tamarindos con suelos de textura arenosa (70–90%) y situados en zonas áridas (Sáhel y zona sudano-saheliana) resultaron ser ricos en propágulos micorrícicos. Las fuertes densidades de propágulos AM en el suelo evaluadas mediante el método del número más probable se hallaron en Niokhoul (1100 propágulos por 50 g de suelo), Sakal (790 propágulos por 50 g de suelo) y en Mbassiss (780 propágulos por 50 g de suelo). Sin embargo la zona agroecológica del Sáhel mostró una colonización micorrícica más alta (11%) que las de las zonas sudano-saheliana y saheliana (3%) del Senegal. **Conclusión.** Nuestro estudio exploró la diversidad natural de los hongos micorrícicos arbusculares; supone un punto de partida para elaborar inóculos aptos para ser empleados en viveros comerciales de producción de tamarindos.

**Senegal / *Tamarindus indica* / micorizas arbusculares / simbiosis / esporas fúngicas / biodiversidad**