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

Runing title: Tamarind parkland mycorrhizal potential in 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 Fabaceae; it is a multi purpose tree with a slow growth. In order to help improving 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: Sahelian zone (i), Sahelo-Sudan zone (ii) and Sudan zone (iii). Soils and roots samples were collected in each site and used for arbuscular mycorrhizal (AM) spores isolation and root colonization assessment. We identified the mycorrhizal fungi from spore collections and evaluated roots mycorrhization rate defined as percentage of roots colonized according to agroecological zones. Results and discussion. Results did not reveal a specific AM fungal strain associated to tamarind plants. Three arbuscular mycorrhizal fungi (AMF) were identified from spores on the genus level: Glomus, Scutellospora and Acaulospora. Tamarinds 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 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 to 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 mycorhizien des parcs à tamarins (*Tamarindus indica* L.) dans trois zones agro-écologiques du Sénégal.

Résumé – Introduction. Le tamarin (*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 des mycorhizes présents dans certains parcs à tamarins au Sénégal. Matériel et méthodes. Trois sites hébergeant une population de tamarins ont été échantillonnés pour chaque zone agro-écologique du Sénégal : la zone sahélienne (i), la zone sahélo-soudanienne (ii) et la zone du soudanienne (iii). Des échantillons de sols et de racines ont été prélevés dans chaque site et utilisés pour isoler des spores de mycorhizes à arbuscules (MA) et évaluer la colonisation des racines. Nous avons identifié les champignons mycorhiziens à partir des spores collectées et évalué le taux de mycorhization 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 tamarin. Trois genres de champignons mycorhizes arbusculaires (AMF) ont été identifiés à partir des spores collectées : Glomus, Scutellospora et Acaulospora. Les sites de tamarins avec sols à texture sableuse (70–90 %) et situés en zones arides (sahel et zone soudano-sahélienne) se sont révélés riches en propagules mycorhiziennes. 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 mycorhizienne (11 %) que celles des zones soudano-sahélienne et sahélienne (3%) du Sénégal. Conclusion. Notre étude a exploré la diversité naturelle des champignons mycorhizes arbusculaires ; c'est un point de départ pour élaborer des inoculums aptes à être utilisés en pépinière commerciale de production de tamarins.

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

The abstract will be translated into Spanish later.

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

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, Ximenia americana*. Furthermore, wild fruit trees are used in traditional medecine 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 Fabaceae 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 on plant co-existence. This knowledge is also needed for appropriate mycorrhization of nursery-grown seedlings for forestation efforts [7]. Current interest in applying low-input in crop production agrotechnology 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 to tropical fruit trees and including tamarind [4]. Previous work has, however, highlighted mycorrhizal dependency of tamarind [5]. The objectives of our study was (1) to characterize the mycorrhizal diversity of rhizosphere associated to tamarind in situ in Senegal through three agro-ecological zones of Senegal, (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 zone in Senegal [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 calliper gauge at (1, 3 and 5) m distance from the trunk, respectively, at a depth ranging 0–40 cm. All samples from one site were mixed into one composite sample and labelled [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, Dakar, Senegal) and another part for arbuscular mycorrhizal fungus spore isolation.

2.1. Tree root colonization

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 roots infection level was estimated using Gridline intersect method [9]. Histological observations were carried out under the microscope (40×) by deposing root fragments (0.5 cm) on a Petri dish under a squared-bottom grid line. Observations were done 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 direction). 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 = (Nmh + Nmv) / (Niv + Nih)], where Nmh and Nmv are mycorrhized root intersections on horizontal and vertical lines, respectively, whereas Niv and Nih 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 along six levels of dilution. Each level of dilution was repeated five times in experimental bucket (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 remain 50 g, 30 g was taken for dilution 10^{-2} ;

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

Zea mays L. seeds were used as 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 d, 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 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 to Tamarindus indica L.

The natural diversity of AMF in the root-associated soil from long-term established tamarind trees was analysed. Soil samples taken from tamarind parklands (*figure 1*) were used for AMF trapping in greenhouse conditions. Two distinct types of inoculums 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 plant were used as mycorrhizae 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 root 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 were extracted following wet sieving method of Gerdemann and Nicholson [13]. For each soil sample, 200 g of soil were used for spore's 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 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 to an internet-published reference culture database established by Morton¹. Spores were compared also 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 fibre 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

Root colonization rate was obtained following formula (F) and data subjected to 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 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 have been evaluated (*table I*). It appears that trees from Sahelian zone are more colonized by mycorrhizae (11.17%) than 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 to 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 stress suffered of several natures (water deficit, low soil fertility level) in arid and dry environments [16].

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

Multiple component analysis on correlation matrices (using Partial Least Squares regression) indicated that the density of viable arbuscular mycorrhizal propagules (MPN), soil pH (water), pH (KCI) and sodium (Na) of the soil are closely related (figure 2). In this diagram, other soil factors, total carbon, 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 on arbuscular mycorrhizal propagules density. Differences in pH are also known to have an impact on spore viability but not on mycorrhizal infection [12]. As previous researches, 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 propagules density [17]. However, sandy soil texture (70-90%) of 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) ($R^2 = 0.93$, $p \le 0.05$). 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. Mycorrhizae 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 to tamarind because we only worked on the genus level (figure 4). Similar results was obtained by various authors [3, 4]; they identified 13 arbuscular mycorrhizal fungi species associated to tamarind specifically in arid area in order to improve water and mineral nutrition. However, in dry area 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 researches [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 against 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 as tamarind parkland. Soil management may influence diversity and roots mycorrhization rate [28, 29]. Definitively, 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 parklands Tamarind.

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Footnote

¹ see <u>http://invam.caf.wvu.sedu/Myc_Info/Taxonomy/species.htm</u>

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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-Sahel	5.72 b
Gnibi	1.5 c		
Mbassis	11.63 ab		
lbel	2.45 c	Sudanian	3.85 b
Welingara	5.12 bc		
Dogoro	3.98 c		

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

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_{H2O}	N (total)	Organic matter %	P available mg•kg ⁻¹	Soil texture (%)		
			(iotai) %	70	Шу•ку	Sand	Limon	Argile
Sakal	Sahel	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-Sahel	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
lbel	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 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).

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.

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 mycorrhizae diversity associated with tamarinds studied in parklands of three agro-ecological zones of Senegal.